QUANTIFYING THE EFFECT OF MICROBIAL DIVERSITY AND PLANT ROOTS ON SOIL STRUCTURAL DEVELOPMENT

BY:

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ABSTRACT

Soil is one of the most complex ecosystems in the environment and changes to microbial diversity are thought to affect the physical structure (and *vice versa*). A fundamental question addressed in this research, is how microbial communities influence the functioning of soil, particularly with respect to the development and maintenance of a soil's physical structure. Using micro- and macrocosms of sieved (and therefore structureless) soil, the effects of time, soil texture, manipulated background microbial diversity, and *Plantago lanceolata* (± mycorrhizal fungi) on the development of soil structure were determined. Background microbial diversity was manipulated using the dilution technique to give a low (10^1) dilution or a high (10^6) dilution of the original soil. This resulted in greater bacterial diversity in the lower (10^1) dilution than in the higher (10^6) dilution when in the presence of mycorrhizal plants. However, background diversity was the same irrespective of dilution in soils with non-mycorrhizal plants (and reversed within the bare soil). Micro- and macrocosms were continually assessed during controlled incubation periods ranging from 7 weeks to 15 months. Of the soil textures analysed (clay loam, loamy sand and sandy loam), loamy sand displayed the highest overall porosity as well as a noteworthy development in porosity throughout the incubation period. Mycorrhizal and non-mycorrhizal plants increased the speed of soil structural development by 5 months relative to unplanted soils. Although mycorrhizal fungi stunted root growth initially, aggregates within mycorrhizal planted treatments were smaller but nonetheless more stable than those in bare soil. Increasing mycorrhizal fungal species richness enhanced root and shoot biomass and reduced aggregate size and total porosity. There was a positive relationship between total porosity and numbers of culturable bacteria and fungi. In soils containing a lower microbial diversity, an increase in porosity, mean pore size, aggregate size and pore perimeter was observed. Results obtained were dependent on incubation conditions, planting regime and mycorrhizal status. Therefore, the effects of reducing microbial species diversity on soil structure parameters are idiosyncratic, with the presence of plant roots acting as a key factor.

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This work is dedicated to my parents and grandparents
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1 INTRODUCTION

1.1 SOIL STRUCTURE

Soil is predominantly composed of single particles (namely sand, silt and clay) that are bound together to form aggregates (groups of particles) of various size and shapes. This process of aggregation (section 1.1.1) influences the structure of soil and subsequently soil function (i.e. the capabilities of the ecosystem for environmental, agricultural and protection processes e.g. nutrient cycling, storage and biological habitat (Karlen *et al.,* 1997)). There is no exact definition of soil structure; however there is universal agreement it should include the arrangement of particles into aggregates, and the size, shape and distribution of pore spaces both within and between these aggregates (Rowell, 1994). Other descriptions also take into account the degree of stability in aggregates (Bronick and Lal, 2005).

The structure of the soil can be separated into different structural grades (Figure 1.1). A structure-less soil consists of individual grains (hence no aggregation) whereas a massive structure is where individual particles form a large cohesive mass (i.e. similar to that found in a compacted soil). In comparison, a strongly developed soil will contain well-formed and stable aggregates that resist collapse or breakdown during disturbance; with little evidence of the individual soil particles, such as that seen within a crumb or granular structure (Figure 1.1). A comparison of the field appearance of a good or poorly structured soil within the field can be seen in more detail in Figure 1.2. In addition to characterisation of an aggregate by shape, assessment can be made through an aggregate's size. Tisdall and Oades (1982)

suggested aggregates can either be micro-structures (i.e. the clay level <2 µm diameter), micro-aggregates (2 - 250 µm diameter) or macro-aggregates (> 250 µm diameter).

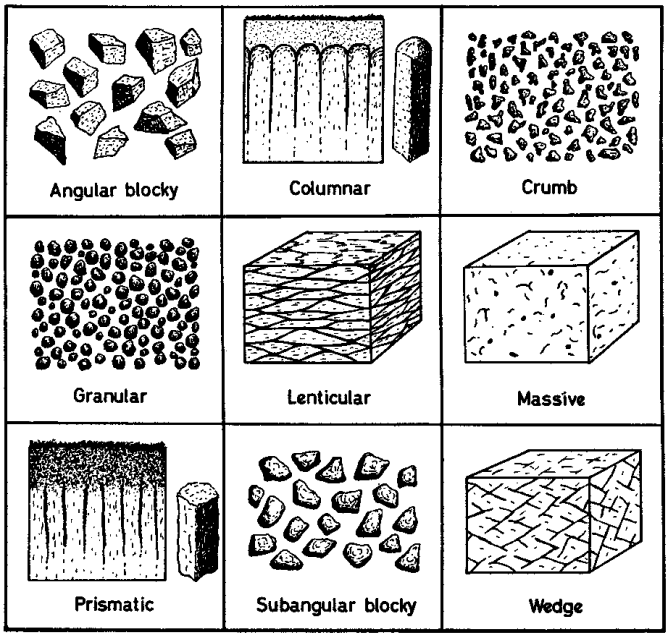


Figure 1.1: Main types of soil structure units / aggregates (Taken from Fitzpatrick, 1986)

Figure 1.2: Field representation of a good and poor soil structure (Figure adapted from Environment Agency "Think Soils" (2008)).
(NB: A good soil structure has clearly defined aggregates with pore space within and between the aggregate, in comparison the poorly structured soil shows a compacted soil environment with little porosity).

Soil structure controls various movements within the soil system including that of water, air and heat flow (National Research Council, 1993). The transport of nutrients, in addition to pollutants to groundwater will be influenced by the structure of the soil. Further affects include the ability of roots to penetrate soil, the extent of soil erosion (since aggregation binds particles together, which would otherwise be susceptible to wind and water erosion), plant growth and subsequent crop yields and finally, microbial communities (Bronick and Lal, 2005). A good soil structure is therefore desirable for sustaining agricultural productivity and for preserving environmental quality. At present, techniques to quantify soil structure are developing and thus facilitating the understanding of processes that take place within such a complex environment.

1.1.1 AGGREGATE FORMATION AND STABILISATION

The formation and stabilisation of aggregates is vital for maintaining a good soil structure. Aggregates, formed by processes given in Table 1.1, can be stabilised, preventing degradation. This occurs mainly through binding agents such as root and fungal mucilage and other microbial exudates that act in a similar manner to cement, binding particles together. These organic binding agents can be classified into three groups; transient, temporary and persistent (Tisdall and Oades, 1982). Transient binding agents are organic materials, in particular polysaccharides, derived from microbes and plants which are readily decomposed. Their effects only last for a few weeks. In comparison, temporary binding agents, from roots and fungal hyphae can last months or even years. Finally, degraded humic material, associated with

amorphous iron, aluminium and aluminosilicates are persistent binding agents (Tisdall and Oades, 1982; Cambardella, 2006).

Table 1.1: The processes that influence soil aggregation (adapted from Bronick and Lal, 2005).

Physical and Biological processes influencing soil aggregation	
Physical processes	**Biological processes**
• Soil texture will influence aggregation as the quantity of clay present in the soil will influence the expansion and shrinkage of soil (Denef *et al*., 2002). • Clay flocculation is a primary agent for aggregation (Dexter, 1988) in addition to the presence of polyvalent metal cations such as Ca^{2+}, Fe^{3+}, Al^{3+}, oxides and hydroxides of Fe and Al. • Drying and wetting cycles form cracks and channels which create and break aggregates (Denef *et al*., 2002). • Freeze thaw cycles lead to cracking that forms and breaks aggregates. • Root movement through soil can orientate and bind soil particles together (Kleinfelder *et al.,* 1992). • Temperature changes affect soil moisture content (Boix-Fayos *et al*., 2001). • Human activity e.g. compaction affects pore size, shape and distribution. • Cultivation affecting aggregate size and stability (Six *et al*., 2000; Denef *et al*., 2002; Pulleman *et al*., 2005).	• Plant growth and particularly root activity removes soil water causing shrinkage of soil, initiating cracking. • Burrowing activities of soil animals will orientate particles bringing them closer together. • Earthworm casting forms aggregates and influences their stability (Scullion, Neale and Philipps, 2002). • Fungal hyphae (in particular mycorrhizal) orientate soil particles, bringing them closer together in a physical network (Bossuyt *et al*., 2001). • Polysaccharide gums and glues from microorganisms aid stabilisation (Amézketa and Aragües, 1995; Czarnes *et al*., 2000). • Plant exudates will affect microbial activity (Traoré *et al*., 2000). • The quantity of organic matter will influence aggregate formation (Denef *et al*., 2002).

Numerous models of aggregation have been described in the literature including that of Tisdall and Oades, (1982); Elliott, (1986); Oades and Waters, (1991); Six *et al. (*1998) and Six, Elliott and Paustain, (2000). All these models suggest the hierarchical order of aggregation with the concept of micro- and macro- aggregates however; they differ in their description of the individual stages of soil aggregate formation and the mechanisms involved. These include humic acids and inorganic ions for microstructures, microbial materials such as polysaccharides, hyphal fragments and bacterial colonies in micro-aggregates and a combination of plant roots, fungi and larger soil fauna in macro-aggregates (Degens, 1997; Carter *et al*., 1999; Czarnes *et al.,* 2000; Schjonning *et al*., 2002; Carter, 2004). This suggests that each sized aggregate unit will have differing stabilities and responses to different environmental stresses (such as rainfall, wind and water infiltration). Macro-aggregates, for example, are readily disrupted by wetting and through gentle agitation, whereas in comparison the smaller micro-aggregates have higher stability making them less susceptible to breakdown unless prolonged and intense agitation is applied. Dexter (1998) used the idea of hierarchical aggregate formation to describe a good soil structure as "one where all the hierarchical orders are well-developed and are stable against the actions of water and external mechanical stress". Such a definition is indeed representative of a good soil structure, but does not consider the importance of the pore spaces between these aggregates.

1.1.2 SOIL POROUS ARCHITECTURE

The shape and size of pore spaces between aggregates can influence the function of the pores (Hattori, 1988) such as water, nutrient and air movement. Macropores (> 50

μm) allow water drainage, aeration and root penetration; whereas micropores of 50-0.2 μm size (normally present *within* aggregates) allow aeration of aggregates and water storage for plant use. In some cases, if micropores are sufficiently small, they will hold water that is unavailable to plants. Some researchers have however, further subdivided pore sizes into classes based on their primary functions within the soil environment (Table 1.2).

Table 1.2: A functional classification of pores based on size. Taken from White (2006).

PORE DIAMETER (μm)	BIOTIC AGENT AND DESCRIPTIVE FUNCTION
5000-500	Created by cracks, earthworm channels and main plant roots. Allows aeration and rapid drainage of soil.
500-30	Created by grass roots and small mesofauna. Allows normal drainage and aeration.
30-0.2	Created by fine lateral roots, fungal hyphae and root hairs. Allows storage of 'available water'.
< 0.2	Created by shrinkage and swelling in clays. Stores residual or 'non available' water.

1.1.3 MEASUREMENT OF SOIL STRUCTURE

To assess soil structure in relation to its function, measurements such as bulk density, porosity, water retention, hydraulic conductivity, aggregate stability, aggregate water repellency and soil thin sections (micromorphology) are typically used. More recently, non-destructive methods that utilise image analysis for the quantification of soil structure have improved rapidly due to technological advancements in both image capturing, manipulation and storage. Development of techniques such as X-ray Computed Tomography (CT) (Macedo *et al*., 1999) and Nuclear Magnetic Resonance (NMR) (Randall, Mahieu and Ivanova, 1997) have further developed the assessment of soil structure through visualisation and analysis.

1.1.3.1 Aggregate stability

The measurement of aggregate stability depends directly on two forces that are applied to a soil aggregate. Those being the binding forces that hold an aggregate together compared to the nature and magnitude of the disruptive forces applied to an aggregate (Amézketa, 1999). As mentioned in section 1.1.1, the aggregate unit size will influence the mechanisms leading to aggregate stability, thus differently sized aggregates will vary in their stability. Micro-structures of clay and silt particles for example will withstand vigorous shaking, whereas larger macro-aggregates of sand for example will not be as resilient. As a result the characterisation of aggregate stability needs to take into account the differences of micro- and macro-aggregates. Micro-aggregate stability is generally assessed through tests focused on dispersion of clay and silt particles when subjected to a wetting and disruptive energy before assessment either optically or densimetrically within an aqueous solution (Rengasamy *et al*., 1984; Piccolo and Mbagwu, 1990). Macro-aggregate stability however, can be determined either through the breakdown of aggregates due to wetting actions otherwise known as slaking (through fast or slow wetting), mechanical action or the combination of both these procedures. A common method developed from previous techniques (Yoder, 1936; Kemper and Koch, 1966; Williams *et al*., 1966; Kemper and Rosenau, 1986) is to determine the size distribution of water wet aggregates to assess the amount of macro-aggregates that have remained stable after the disruptive event (Jastrow and Miller, 1991; Le Bissonnais, 1996). All techniques have undergone variations, influenced mainly by sample collection and preparation (including the water content of aggregates tested), soil type, equipment availability, and measurement technique that have led to the lack of a standardised methods for

aggregate stability assessment. Consequently, it has become widely acknowledged that an unified approach for micro- and macro- aggregate stability measurement needs to be proposed that would allow simple, easy and effective techniques to provide comparative data sets between various research studies.

1.1.3.2 Aggregate water repellency

The method for measuring the hydraulic properties of individual aggregates was first designed by Leeds-Harrison, Youngs and Uddin (1994). This method described a simple and rapid approach to allow convenient replication for the assessments of the micropore region within aggregates. Further adaptation by Hallett and Young (1999) allowed the examination of the water repellent characteristics of soil aggregates. Subcritical water repellency of soil is where water uptake appears to occur readily, yet it is impeded to some extent, due to hydrophobic surface films (Hallett, Baumgartl and Young, 2001). It is a common state of soil, and from recent research it has been acknowledged that subcritical water repellency has both beneficial and detrimental effects in the soil environment and on agriculture. If a soil has a slight subcritical repellency, the speed of water infiltration is reduced and hence soil aggregates are not subjected to slaking (see section 1.1.3.1), however the disadvantage of water repellency may include increased surface runoff, resulting in the loss of soil particles from the surface and even the transport of pollutants to more vulnerable ecosystems. The water repellency of a soil is thought to be modified due to microbial activity (Hallett and Young, 1999); fungal biomass (Feeney, 2004); agricultural management (Hallett, Baumgartl and Young, 2001) and plant waxes (Neinbuis and Barthlott,

1997), in addition to other factors (such as organic matter, soil temperature and fire) described in the review by Doerr, Shakesby and Walsh (2000).

1.1.3.3 Soil micromorphology

The use of thin-sections and image analysis are extremely important regarding the assessment of soil structure and interpretation of the spatial location of bacterial cells. Thin sectioning allows a high resolution assessment of the soil environment (up to resolutions of < 2 μm when scanning electron microscopes (SEM) and transmission electron microscopes (TEM) are used (Bullock *et al*., 1985; Schaap and Lebron, 2001)) that maintains the spatial context of pore networks. Numerous investigations have been undertaken using thin sections to determine soil structure (FitzPatrick, 1986); pore architecture (Moran *et al.*, 1988; Drees *et al*., 1994); pore size distributions (Tippkötter *et al*., 2009); root-soil contacts (Kooistra *et al*., 1992); water movement and aggregate stability of different management practices (Pagliai, Vignozzi and Pellegrini, 2004).

Otten *et al*. (2004) highlighted the importance of macropore structure in soil on the parasitic spread and saprotrophic invasion of soil by *Rhizoctonia solani*, whilst the use of biological stains in thin-sections has allowed determination of the location of bacterial cells within soil (Nunan *et al*., 2001; Harris *et al*., 2002). Furthermore thin sections have also assessed the impact earthworms and their burrows have on soil structure (Ligthart, 1997). Such visualisation is vital in understanding the interaction of soil biota within the soil matrix even though the process is limited to 2-D compared to that of X-ray Computed Tomography (section 1.1.3.4). The recent development of

techniques allowing the visualisation of microbial cells within thin sections has provided an insight into microbial preferences within their natural physical habitat (Figure 1.3).

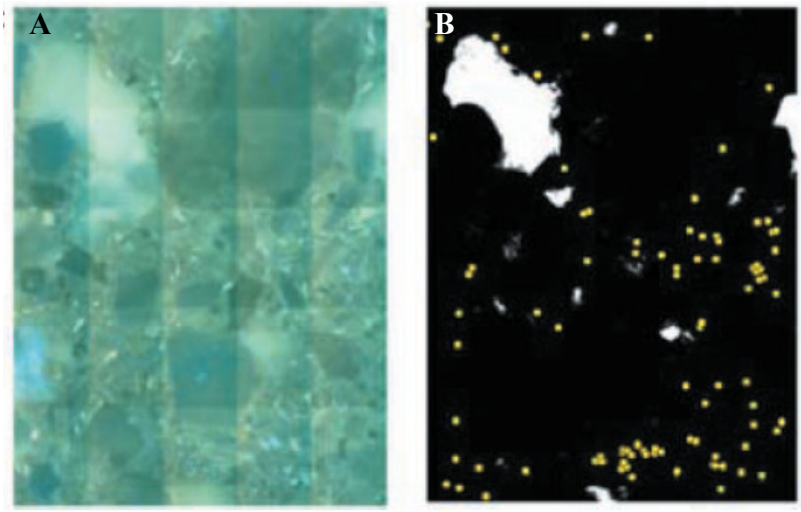


Figure 1.3: Example of a high resolution biological thin section taken from Young and Crawford (2004).
Fluorescently stained microbial cells are observed under an epifluorescent microscope (A). After a series of image analysis steps an image can be produced to show the location of microbial cells (yellow) within the soil matrix with pore spaces represented as white and the solid black (B).

1.1.3.4 X-Ray Computed Tomography

In recent decades, the use of image analysis to define and quantify soil structure (Ringrose-Voase and Bullock, 1984; Ringrose-Voase, 1987; Ringrose-Voase, 1996; Vogel, 1997; Horgan, 1998; Pierret *et al*., 2002; Lontoc-Roy *et al*., 2006; Luo, Lin and Halleck, 2008) has increased rapidly, mostly due to advances in digital cameras, high resolutions, higher storage capacities and faster processors and computers and through advancements of X-ray Computed Tomography (CT). X-ray CT is a non-destructive and non-invasive method which creates relatively rapid images of the porous media of soil particularly compared to that of thin sections. Analysis of these

images allows thorough assessment of the soil pore network in addition to aggregate development. The use of X-ray CT within soil science was first described by Petrovic, Siebert and Rieke (1982) who assessed the relationship between bulk soil density and X-ray attenuation.

The theory behind the use of X-ray CT, based on photon emission, has been covered in numerous reviews (Van Geet, Swennen and Wevers, 2000; Mees *et al*., 2003; Cnudde *et al*., 2006; Taina *et al*., 2008). Briefly X-ray CT uses X-rays that intersect the soil column perpendicular to its longitudinal axis, producing images of slices through the soil core. Images of the soil are created due to differing attenuation of the X-ray radiation by various features in the soil, reflecting the density of those features present due to interactions with the constituent atoms (Taina *et al*., 2008). The attenuation is due to three mechanisms namely incoherent scatter (affected by density of material scanned), coherent scatter (redirection of X-ray photons without the loss in energy) and photoelectric absorption (resultant of proton absorption within an atom and the ejection of an electron (Kak and Slaney, 1988; Simons, Verhelst and Swennen, 1997)). The generated image is the matrix of voxel (volume units for pixels) numbers expressed in Hounsfield units (HU). These values in turn relate to the density of the materials assessed. In general, pore spaces are associated with low densities, for example, a value of 0 HU would represent distilled water and -1000 HU represents air (at standard temperature and pressure) (Taina *et al*., 2008), whereas mineral materials are of higher density and would have a higher value. An example of an X-ray CT image of soil can be seen in Figure 1.4, where pore space is represented in black and soil material in grey. X-ray CT projections that are attained by reconstruction consist of linear integration of the attenuation coefficient, the most

common technique being the filter back-projection algorithm (Kak and Slaney, 1988). The accuracy and quality of images analysed from X-ray CT however is highly dependent on the quality and resolution of the initial image acquired (Bui, 1991).

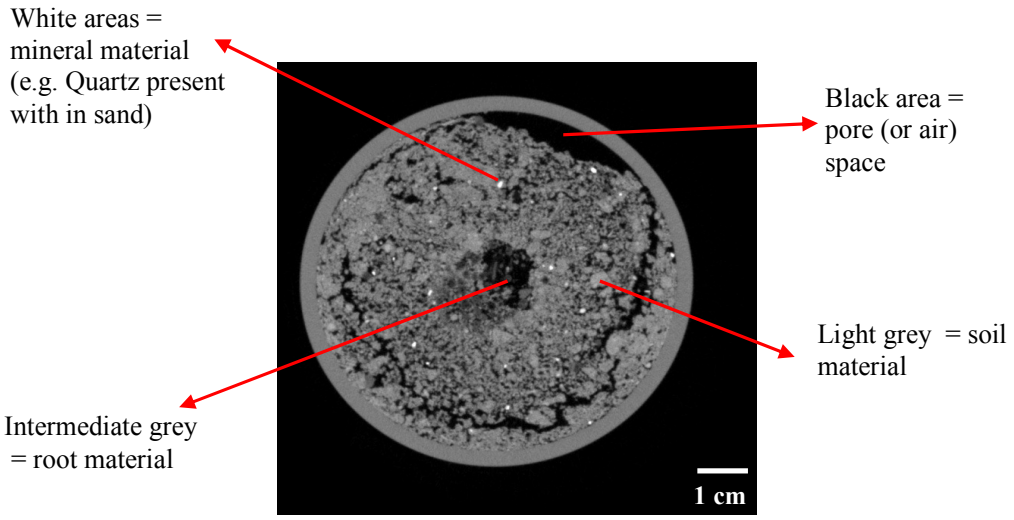


Figure 1.4: Example of an image taken using X-ray CT scanning.

X-ray CT scanning in soil science has great potential for structural visualisation in both 2-D and 3-D, where previously only 2-D visualisation was possible with the use of thin sections. The use of X-ray CT has improved and also allowed invasive determination of macropores down to diameters of 0.5–1.0 mm (Warner *et al*., 1989; Anderson, Peyton and Gantzer, 1990), in addition to tortuosity, hydraulic radius, numerical density, pore connectivity, macropore size, distribution, length and branching from mathematical morphology parameters (Perret *et al*., 1999; Pierret *et al*., 1999, 2002). Close examination of preferential flow patterns was undertaken by Heijes, Ritsema and Dekker (1996) who reported preferential flow within soil was determined by macropore networks. Such work was developed further by Mooney

(2002) who quantified water infiltration using repeated scans after an infiltration period producing a 3-D map of pore space and water movement.

Other applications have included the assessment of biological interactions within the soil environment. Nunan *et al. (*2002) assessed the effect of pore geometry with regard to micro-aggregates and microbial habitats using thin sections, but through the use of X-ray CT a later investigation (Nunan *et al*., 2006) discovered that the 3D architecture of micro-aggregates was directly related to the scale of microbial habitats of fungi, bacteria and other microbiota. In addition, Johnson, Read and Gregory, (2004) used X-ray CT to track the movement and final position of clover root weevil larvae in real time within the soil environment. Further studies have also been undertaken to assess the effect of earthworms on soil structure (Joschko *et al*., 1991, 1993). Capowiez, Pierret and Moran (2003) used X-ray CT to report that earthworm burrows vary with season, work that was further developed by Bastardie, Capowiez and Cluzeau (2005) who discussed the potential impact earthworms have on soil function after assessment using X-ray CT.

Root systems are very difficult to quantify, particularly non-invasively due to their complex morphology (Gregory, 2006), but more importantly since they grow in a medium of soil that is both opaque and very difficult to handle. As a result numerous attempts using X-ray transmission imagery, nuclear magnetic resonance (NMR) and X-ray CT have been undertaken to assess the impact roots have within the soil environment non-invasively (Rogers and Bottomley, 1987; Brown *et al*., 1991; Pierret *et al*., 1999; Gregory *et al*., 2003). Through the use of X-ray CT, root diameter and length have been measured (Gregory *et al*., 2003), the spatial organisation of tree

roots (Pierret *et al*., 1999) and drawdowns in soil water content associated with radish roots (Hamza, Anderson and Aylmore, 2001). Jenneson *et al. (*2003) also described the use of a low dose X-ray CT machines for root imagery that would reduce any undesirable effects on X-ray CT on plants, their roots and the microbiota within the soil environment, whilst Thieme, Schneider and Knöchel (2003) described the use of X-ray nanotomography to examine the 3D structure of flocs of colloidal particles and the spatial arrangement of bacteria within them.

1.2 SOIL MICROBIOLOGY

Soil remains one of the most uncharacterized ecosystems in the environment, despite it being the vital link between biotic and abiotic components of the terrestrial ecosystem. It is widely acknowledged that soil systems are extremely diverse and complex with a large biodiversity (Giller *et al.,* 1997; Torsvik and Øvreås, 2002; Fitter, 2005; Fitter *et al*., 2005) even if this is uncharacterized. The soil environment consists of mineral particles that vary in size, shape and chemical characteristics, plant roots, organic matter, gases, water, dissolved minerals and living biomass. The microbes that exist within the soil are vital for the maintenance of functions (such as decomposition and nutrient availability) in addition to the preservation of soil quality.

1.2.1 THE SOIL MICROBIAL COMMUNITY

The most diverse members within the soil community are microbes (i.e. bacteria and fungi). There are also many other animal species that live within the soil including

microfauna (body width < 0.1 mm; e.g. protozoa and nematodes), mesofauna (body width 0.1–2.0 mm; e.g. microarthropods and enchytraeids) and the macrofauna (body width > 2mm; e.g. earthworms and millipedes) (Bardgett, 2005). The dominating organisms in soils, in terms of total biomass are fungi with as much as 250 kg ha^{-1} dry fungal hyphae within the top 5 cm of soil, however in terms of total numbers and diversity, bacteria form the largest proportion of the soil microbial community (Bardgett, 2005).

There are many estimates suggesting the number of bacteria inhabiting soil, including that of Atlas and Bartha (1987) who suggested 10^6 to 10^9 bacteria within a gram of soil. More recent estimates have been more conservative suggesting 10^4 and 10^6 bacterial species are present in one gram of soil (Torsvik, Goksøyr and Daae, 1990; Gans, Wolinsky and Dunbar, 2005). With such high bacterial numbers present within the soil environment, the importance of the functions these organisms perform within the soil ecosystems is vital to plant growth, nutrient cycling, soil structure and maintenance of soil productivity. Furthermore the functioning of these organisms will be controlled by interactions such as mutualism, commensalism, antagonism, competition, parasitism/predation and neutralism with each other, since such large numbers of microorganisms coexist (van Elsas *et al*., 2007).

1.2.2 ECOSYSTEM FUNCTIONING AND SUSTAINING SOIL QUALITY

Over the last 20 years there has been a loss in biological diversity due to habitat destruction, over-harvesting, pollution and the introduction of foreign plants. As soils are such an important part of the majority of terrestrial environments and to the

success of sustainable agriculture, it is vital that soil quality is maintained and safeguarded (Doran and Zeiss, 2000). Soil quality is defined as the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Doran and Parkin, 1994; Karlen *et al*., 1997).

The soil's role in sustaining plant growth and biological activity is based on the physical structure of the soil (i.e. porosity, pore connectivity, water holding capacity and stability) in addition to chemical and other soil conditions such as organic matter content, nutrient supply, pH, water content and temperature. Soil is an inorganic store in the flow of nutrients within the biosphere; hence soil microbial processes that take place within the ecosystem are essential for biogeochemical cycling (White, 2006). Essential components and processes of the global C, N, P and S cycles take place within the soil by microbes, in addition to other micronutrients (e.g. Fe, Zn, Cu) making the ecosystem an extremely vital part of the whole biogeochemical cycling process. With 5-20 % of the species within a group of organisms having already become extinct, through human activity, that represents a 100-1000 times higher extinction rate than that observed pre-humans (Pimm *et al.*, 1995; Chaplin *et al*., 2000). The importance of determining the impact of soil diversity losses on soil ecosystem functioning is therefore vital for the maintenance of soil quality for the future.

1.2.3 SOIL MICROBIAL DIVERSITY, ECOSYSTEM FUNCTIONING AND REDUNDANCY

Microbial diversity is defined as the "variety of microorganisms at the genetic, species and ecosystem level; the ecological complexes in which they occur, and the ecological processes of which they are part" (Bull, 1992). The diversity of soil biota is important for sustaining soils and particularly within the agricultural system, since microorganisms perform diverse ecological services including recycling of plant nutrients, maintenance of soil structure, detoxification of noxious chemicals and the control of plant and animal pests. Ecosystem function is defined "as the minimum set of processes that ensure the biological productivity, organizational integrity and the perpetuation of the ecosystem" (Swift, Izac and van Noordwijk, 2004).

Generally understanding of microbial diversity within soils is scarce, despite advancements from cultural based plate counts methods towards molecular techniques. Despite these changes, knowledge of the structure of soil bacterial communities is still limited predominantly due to the unculturability of numerous microbial cells within the soil (Torsvik, Goksøyr and Daae, 1990); the inaccuracies of DNA identification, since bacteria are known to exchange DNA within and between species and genera (Davidson, 1999); the sensitivity of microbes to changes in environmental conditions such as drying (Linn and Doran, 1984; Young and Ritz, 2000) and finally due to microbial populations changing over time due to succession and also with space. These limitations in accurate determination of microbial diversity are also affected by the fact that present assays for measuring microbial functions determine the overall rate of entire metabolic processes (such as respiration or specific enzyme activities), without the identification of the actual microbial species involved

(Nannaiperi *et al*., 2003). Such problems in determining microbial diversity and functioning therefore highlight the reason for the lack of knowledge between biodiversity and ecosystem function, particularly within soil where < 1% of microorganisms observed under a microscope are cultivated and characterised (Torsvik and Øvreås, 2002). Therefore it is key to understand the relationship between genetic diversity and community structure and between community structure and function (O'Donnell *et al.,* 2001).

Despite no clear relationship between biodiversity and functioning established for the soil ecosystems, it is clear that within plant ecology, a relationship has been established between the number of species and biomass produced (Tilman, 1999; Loreau *et al.*, 2001). These studies highlighted that plant growth increases with the number of species up to a threshold maximum. Some studies however, have highlighted the impact biodiversity has on soil functioning. Naeem *et al*. (1994) predicted that benefits to ecosystem function were derived from higher biodiversity, paving the way for the development of the insurance hypothesis (Yachi and Loreau, 1999). This proposed that biodiversity buffers ecosystem processes against environmental change because different species or phenotypes respond differently to these changes resulting in functional compensation and more ecosystem properties.

Griffiths *et al. (*2000, 2001) showed the effect of microbial diversity on soil ecosystem functions depended on the specific function measured. Functions such as substrate induced respiration (SIR) increased with decreasing microbial diversity; whereas others were not induced (such as thymidine and leucine incorporation which measures microbial activity, NO_3^- accumulation and respiratory growth response);

whereas others declined when microbial diversity was lower (such as C-substrate utilisation (Biolog), short term respiration and potential nitrification rates). Some research has further studied the effect microbial diversity has on organic matter decomposition, with Chander *et al.* (2002) reporting that soil fumigated with chloroform (with a much smaller microbial biomass than the non-fumigated soil), respired the same quantity of ^{14}C-CO_2 from labelled straw as the non-fumigated soil. Griffiths *et al*. (2000, 2001) and Nannipieri *et al.* (2003) found no relationship between microbial diversity and decomposition of organic matter existed. Degens (1998) however, suggested no conclusions could be drawn from assessing the effect of reducing or increasing the catabolic diversity of microbial communities on organic matter decomposition, particularly since soil moisture conditions influenced the results.

While the significance of species diversity on soil function is unclear, there are three classes of biodiversity–functioning hypothesis that were described by Naeem, Loreau and Inchausti (2002) (Figure 1.5). Firstly, species are "redundant" (i.e. the loss of species is compensated for by other species, or the addition of new species to an ecosystem adds nothing new to the system). Secondly, species may be primarily "singular". This hypothesis implies that each species contributes to ecosystem functioning uniquely, hence their loss or addition causes detectable changes in functioning. A keystone species is an example of how one species can have a significant effect on ecosystem functions. The final hypothesis is that species impacts are "context-dependent" and therefore idiosyncratic where the impact of loss or addition of a species depends on the ecosystem conditions (e.g. community

composition, soil fertility, substrate availability) under which the local extinction or addition occurs.

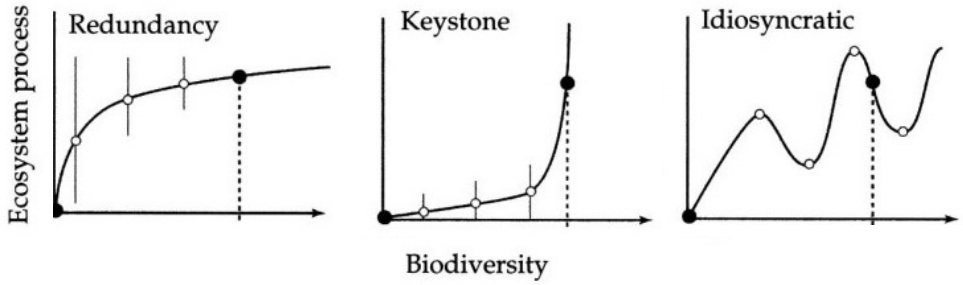


Figure 1.5: Graphical representation of the hypothetical relationships between biodiversity and ecosystem processes (Adapted from Naeem, Loreau and Inchausti, 2002).

1.2.4 MYCORRHIZAL FUNGI

Fungi play an important role in the recycling of important chemicals that would otherwise remain locked up within detritus. Fungi are primary organisms that cannot synthesise their own food and are dependent on complex organic substances for their carbon. Specialised fungi can be pathogenic to plant tissues, while others can form mutually beneficial relationships with plants and assist in direct nutrient supply to plant cells. One particular group of fungi in the soil, mycorrhiza form symbiotic relationships with plant roots. Mycorrhizal fungi can be separated into six common types; Arbuscular, Ecto, Ericoid, Arbutoid, Monotropoid and Orchid (Smith and Read, 1997).

This review will focus on one of the most common type of mycorrhiza the Arbuscular mycorrhizas (AMF). Initially AMF were believed to form mutualistic associations with > 70-80 % of plant families (Newman and Reddell, 1987; Trappe, 1987),

including the majority of domestic and wild plant species, whereas species such as pine, firs and spruces are not colonised. Recent studies however, have since reported symbiosis can be as high as 90 % of plant families (Clapp *et al.*, 2002) which is similar to that found by Koske, Gemma and Flynn, (1992) who reported that > 90 % of the endemic Hawaiian plant species consistently formed mutualistic relationships with AMF. Such research highlights the true extent of their distribution.

Mutualistic relationships are formed within soil as AMF germinate and colonises the growing roots of the first compatible host. Once AMF have penetrated the roots and established internal structures that allow the fungus to obtain carbon substrates from the plant, extraradical mycelium will radiate from the colonised root. Internal structures include arbuscules that act as sites of nutrient and carbon exchange between the symbionts and vesicles acting as sites of lipid storage for the AMF. Eventually an extensive extraradical mycelial network will develop within the soil, colonising other neighbouring host plants, exploring the soil for new colonisation sites in addition to absorbing nutrients. Individual AMF are not host specific, which means that a single AMF species can grow in the roots of most plant species, however the mechanisms of establishment and ultimately their function may vary for different AMF species and genera (Dodd *et al*., 2000).

AMF are abundant within a large range of ecosystems from wetlands (Wolfe *et al.*, 2007) to agricultural systems (van der Heijden *et al*., 2008) and waste sites contaminated with zinc (Turnau *et al.*, 2001). Plants will support the AMF by supplying carbon derived from photosynthates. On the other hand AMF will facilitate plants by protecting them from drought and improving their water efficiency (Smith

and Read, 1997; Al-Karaki, McMichael and Zak, 2004; Finley, 2004; Abo-Ghalia and Khalafallah, 2008), protection from pathogenic attack (Newsham, Fitter and Watkinson, 1995a, b) as well as providing vital nutrient uptake sites for plant-limited nutrients such as phosphorus (Smith and Read, 1997). The transport of phosphorus through AMF hyphae can be six times faster than simple diffusion of phosphorus through soil to plant roots (Bolan, 1991). It has even been suggested that with plants colonised by AMF, the fungus becomes the primary method for nutrient uptake. It is worth noting however, that some mycorrhizal fungi can be parasitic if plant roots are colonised to an extremely high degree, as they will eventually drain the plants of their resources, taking up to 20 % of the plant's total carbon budget (Jakobsen and Rosendahl, 1990).

AMF can bring many benefits to the soil including carbon sequestration (Treseder and Allen, 2000) in addition to enhanced plant growth and improved crop yield under drought conditions (Plenchette, Furlan and Fortin, 1981; Morin *et al*., 1994; Wu, Xia and Zou, 2008). AMF can also influence plant diversity (Gange, Brown and Farmer, 1990; van der Heijden *et al.*, 1998a, 1998b; Hartnett and Wilson, 1999) leading to a more diverse ecosystem, with increased organic matter content and improved nutrient cycling. Plant species also influence AMF diversity within a soil ecosystem (Helgason *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002, 2003; Johnson *et al.*, 2003). Furthermore, AMF will also improve plant productivity within systems with minimal numbers of plant species (Kilironomos *et al.*, 2000). The ability of AMF to stabilise soil structure will be discussed in section 1.3.2.

1.2.5 TECHNIQUES FOR STUDYING SOIL MICROORGANISMS

Current knowledge of soil biota is restricted as there is still no accurate or effective method to determine levels of soil biodiversity within soil. It is widely acknowledged that we are ignorant about the species that live in the soil; this is not aided by previous inappropriate culture techniques (Tiedje *et al*., 1999; Sait, Hugenholtz and Janssen, 2002; Gomez, Garland and Roberts, 2004; Fitter, 2005), resulting in estimates of culturable species being < 5 % and even as low at 1 % (Amann, Ludwig and Schleifer, 1995) of the total microbial community. Since this is the case more accurate extraction and cultivation methods are required (similar to those described by Joseph *et al. (*2003)). However, it is not just microbes that are still uncharacterized; information regarding microarthropod communities is also restricted (André, Ducarme and Lebrun, 2002).

Traditional techniques for studying microorganisms include plate counts, a culture dependent technique that is fast and inexpensive. The method provides data on the active population present within the soil, however it is limited since it is culture dependent, with only 0.1-1 % of the soil population cultured. This is because the conditions of incubation restrict the growth of some organisms as the method is nutrient, temperature, pH and light restricted with bias towards the fast growing individuals. In addition to plate counts, many other techniques have been used such as microscopic counts using fluorescent dyes, fumigation-incubation techniques (Jenkinson and Powlson, 1976a, b), fumigation–extraction techniques (Vance, Brookes and Jenkinson, 1987) and substrate-induced respiration (Anderson and Domsch, 1978; Lin and Brookes, 1999). All of these methods have associated benefits

and disadvantages; however all allow overall population changes to be detected. Molecular techniques allow DNA community profiling to be undertaken; allowing genetic fingerprints of soil microbial communities to be made. These techniques generally make use of polymerase chain reaction (PCR) a technique used to amplify pieces of DNA during repeated cycles of denaturing, primer annealing and DNA polymerase-catalyzed elongation of strands. There are various molecular techniques used, most of which have been discussed in the reviews by Kirk *et al*. (2004) and Liu *et al*. (2006). Advantages and disadvantages also exist for molecular methods, and although PCR-based techniques overcome the problems of culture-based methods, they can suffer from problems associated with bias (i.e. unequal amplification or cloning efficiency (Acinas *et al*., 2005)).

1.2.5.1 Soil microbial biomass

The widely acknowledged methodology for determining soil biomass through chloroform fumigation-incubation was first described by Jenkinson and Powlson (1976b) preceding earlier studies on the biocidal effects on soil microorganisms (Jenkinson, 1966; Jenkinson and Powlson, 1976a; Jenkinson 1976). This method remains the standard procedure to measure biomass, however in some laboratories the principal methodology has now become the fumigation–extraction method (Vance, Brookes and Jenkinson, 1987).

The fumigation of soil with $CHCl_3$, followed by the extraction with a salt solution, has since allowed the measurement of microbial-S (Saggar, Bettany and Steward, 1981), -P (Brookes, Powlson and Jenkinson, 1982; Hedley and Stewart, 1982), -N

(Brookes *et al*., 1985) and most importantly microbial-C (Vance, Brookes and Jenkinson, 1987). Further modification of the method used to assess microbial-C was described by Wu *et al. (*1990) who used automated analysis of organic carbon.

1.2.5.2 Soil microbial community and activity

Garland and Mills (1991) first described a method for determining the metabolic potential of the soil microbial community by use of Biolog® microtitre plates. The Biolog system was first developed for use in the pharmaceutical industry, but was later applied for studying whole environmental microbial communities in soil, aquatic and rhizospheric systems (Bossio and Scow, 1995; Bååth *et al*., 1998; Yao *et al*., 2000; Bundy, Paton and Campbell, 2002). Each microtitre plate consists of 96 wells, each (apart from one that acts as a blank) contains a different carbon substrate in addition to a tetrazolium violet dye. As a substrate is utilised in each well, during incubation, the tetrazolium dye becomes reduced, causing the dye to turn purple. The colour intensity within the well is measured using a microtitre plate reader with an appropriate filter (590 or 595 nm). The patterns within profiles can be used to interpret the differences in the major active members of the microbial community. Such tests have become a popular way to assess changes in community structures and functional diversities (Garland and Mills, 1991; Zak *et al*., 1994) as they are rapid, inexpensive, simple and yield vast details about the functioning of microbial communities within the particular system.

1.2.5.3 Soil microbial community and relative abundance

Terminal - Restriction Fragment Length Polymorphism (T-RFLP) is just one of many community analysis protocols, all which vary in methodology as described in the reviews of Kirk *et al. (*2004) and Liu *et al. (*2006). It is one of the most widely used genetic fingerprint techniques for bacterial ecology studies, as it is an extremely powerful and rapid tool for assessing species richness and the population sizes of communities (Marsh *et al*., 2000) in addition to tracking spatial and temporal changes in microbial diversity. T-RFLP was one of the first techniques to take advantage of automated sequencing gel electrophoresis allowing high reproducibility between samples. T-RFLP analysis is a direct DNA profiling method that usually targets the rDNA (Lukow, Dunfield and Liesack, 2000). It provides a profile of microbial communities through the use of oligonucleotide primers (one of which is labelled with a fluorescent tag) for PCR amplification before the digestion of the PCR products with one or more restriction enzymes (Figure 1.6). The labelled products (otherwise known as terminal restriction fragments (T-RFs) vary in length depending on the DNA sequence of the bacteria/fungi present and the point at which the selected enzyme cuts the sequence. Profiles of T-RFLP are obtained by separation of T-RFs through high resolution gel electrophoresis using automated DNA sequences. The laser scanning system of the DNA sequencer detects the labelled primers (Sakai *et al.,* 2004) and from the dye signal, fragment size (determined through the observation of peaks that relate to one genetic variant within the original sample) and relative abundance of each fragment length (determined through the height of the peak) is given. A downside of the method, as with most molecular techniques is that it relies on the efficiency of lysing and extraction of DNA as well as PCR biases.

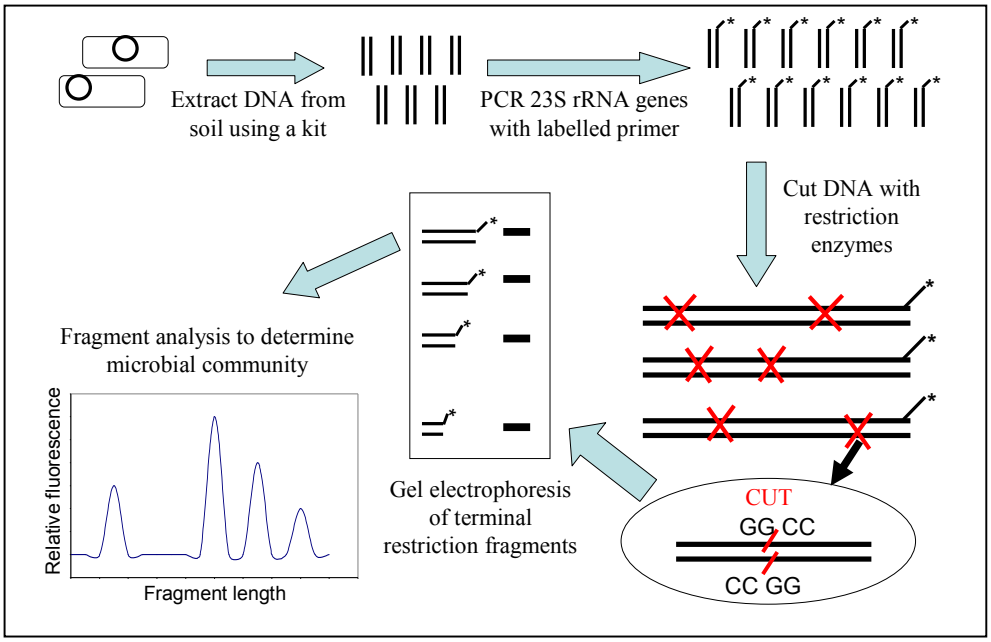


Figure 1.6: Break down diagram showing the processes involved in T-RFLP of bacterial samples.

Previous work has generally focused on the 16S ribosomal DNA (rDNA) since it is highly conserved (Liu *et al*., 1997; Clement *et al.,* 1998; Felske *et al*., 1999; Nunan *et al*., 2005) or the 16S-23S rDNA spacer region since the spacer region is highly variable (Bacot and Reeves, 1991; Barry *et al*., 1991) within many species for bacterial primers. More recently the analysis of the 23S rDNA subunit has illustrated that this region shows more variation between species than the 16S rDNA region (Anthony, Brown and French, 2000). The research focus in fungal primers has been on the small-subunit (SSU) rDNA and the internal transcribed spacer (ITS) region of the rDNA genes. Comparison of these two rDNA regions for T-RFLP analysis in fungal communities was assessed by Lord *et al.* (2002) who discovered a greater fungal diversity within the ITS region, in addition to a lack of specificity of primers within the SSU rDNA region. This highlighted the advantages of amplifying the ITS

rDNA region for T-RFLP analysis, that is now used widely by many researchers (Dickie, Xu and Koide, 2002).

1.2.5.4 Mycorrhizal colonisation and dependency

The measure of plant colonization by mycorrhizal fungi has generally employed the use of a range of stains including trypan blue (Philips and Hayman, 1970; Koske and Gemma, 1989); chlorozal black E (Brundrett, Piche and Peterson, 1984), acid fuchsin (Kormanik and McGraw, 1982) all of which are possibly carcinogenic compared to using ink and vinegar (Vierheilig *et al.,* 1998). Gange *et al.* (1999) highlighted that between 1992-1998 ~ 95 % of methods in arbuscular mycorrhizal literature used staining techniques to determine mycorrhizal colonisation, compared to ~ 3 % that used autofluroscence (Ames *et al.,* 1982). However with such different stains, variations in visualization of individual AMF species vary, producing very different colonization rates, even within the same plant (Gange *et al*., 1999). In addition to measuring root colonisation by mycorrhizal fungi, assessment can also made regarding the degree of dependency the plant has on the colonisation (i.e. if it increased productivity, the plant's maximum growth or yield at a given soil fertility (Gerdemann, 1975)). A wide range of dependencies have been highlighted using this calculation particularly identifying plant species that are never mycorrhizal and hence have no mycorrhizal dependency (MD) (Baylis, 1975). The MD can vary greatly from one plant species to another and even within species (Menge, Johnson and Platt, 1978; Azćon and Ocampo, 1981; Tawarata, Tokairin and Wagatsuma, 2001). Furthermore, MD is influenced by differences in phosphorus availability levels within soils (Mosse, Hayman and Arnold, 1973; Krishna and Bagyaraj, 1982; Habte and Manjunath 1987),

soil type (Mosse, 1972; Daft and Hacskaylo, 1977), soil nutrient levels (Menge, Johnson and Platt, 1978) and between and within mycorrhizal species (van der Heijden, 2002; Oliveira *et al*., 2006). Alternative indicators for mycorrhizal dependency have been used that include morphological root properties including root geometry, rate of root growth, density and length of root hairs (Plenchette, 1991).

1.3 EFFECTS OF MICROBES AND ROOTS ON SOIL STRUCTURE

The link between soil microorganisms and soil structure has been described by Young (1998) as being two-way. All biota within the soil influence the physical structure of the soil by disturbance or by changing it indirectly by exuding gluing agents and C substrates. This in turn modifies the dynamics and transport (through changes in pore connectivity and water flow) of the microorganisms. The following sections below give detailed explanation of the effects microbes, roots and mycorrhizal fungi have on soil structure.

1.3.1 EFFECT OF SOIL MICROBES ON SOIL STRUCTURE

The zone of soil that surrounds roots is called the rhizopshere, a term first devised by Hiltner (1904) who used it to describe the interactions between bacteria and legume roots; whereas the soil zone influenced by just AMF mycelium and soil interactions is known as the hyphosphere (Marschner, 1995). The production of exudates by plants and microbes within the rhizosphere in particular influences soil structure. Bacteria within the soil are known to release exopolysaccharides, high-molecular-weight polymers, containing sugar residues. Czarnes *et al. (*2000) modelled the impact of such exopolysaccharides on soil structure finding that porosity and tensile strength of

the soil increased with its presence. Further studies by Amellal *et al. (*1998) also highlighted that bacterial exopolysaccharides (combined with wet/drying of soil) improved soil aggregation and macroporosity.

Other investigations have highlighted the impact total bacterial numbers have on soil structure. Aşkin *et al. (*no date) illustrated inoculation of soil with additional bacteria increased soil aggregation, however they recommended that in order for this impact to be effective over a long-term period addition of energy materials for bacteria would be required. Since bacteria utilise organic residues within the soil ecosystem, including root and other microbe exudates, their impact on soil structure has been recognised as being short-term or transient (Tisdall and Oades, 1982) as microbes readily decompose exudates that aid initial soil binding.

1.3.2 EFFECT OF ROOTS ON SOIL STRUCTURE

The impacts roots and associated microorganisms have on soil physics and geochemistry were discussed in the recent review by Hinsinger *et al. (*2009). Briefly however, the polysaccharides released by roots (otherwise known as exudates) have been recognised to change the chemical and physical properties of the surrounding soil, modifying soil water release characteristics, hydraulic conductivity, nutrient adsorption, nutrient availability and microbial turnover (Read *et al*., 2003; Gregory, 2006). These exudates act as substrates to many soil organisms, resulting in increases in microbial community composition and hence microbial activity within this rhizospheric region. These in turn influence soil structure indirectly, by generating adhesive forces that stabilise aggregates and influence the water sorptivity and

repellency of aggregates (Czarnes *et al.*, 2000; Traoré *et al.,* 2000). Morel *et al. (*1991) highlighted the true extent these exudates have on aggregate stability, observing a 40 % increase in stability due to maize root exudates. Furthermore, on a long-term scale, root activity within the soil generally increases the organic matter content in the soil, which is known to increase aggregate formation and stability; hence roots indirectly influence soil structure through these organic amendments that in turn influence microbial activity within the soil (Six *et al.,* 2004). In addition to the chemical changes associated with these exudates, roots also influence soil structure through their physical activity within the soil. As roots penetrate through the soil, they create compressive and shear stresses that result in the compression of soil within the roots' vicinity (Dexter, 1987; Braunack and Freebairn, 1988; Hinsinger *et al.,* 2009) resulting in decreased porosity within that region (Bruand *et al*., 1996). Outside of this rhizospheric zone, the effect of roots on soil structure is different, with roots resulting in the enlargement of existing pores and the creation of pores (Angers and Caron, 1998). Furthermore root activity also results in soil fragmentation, resulting in zones of failure causing the destruction of macro-aggregates to micro-aggregates and loosening of the soil structure. This breakdown of macro-aggregates however may induce aggregation according to Tisdall and Oades (1982) who highlighted micro-aggregates were important in the hierarchy of soil structure development. Whilst root activity is known to influence the porous nature of the soil though their movement within the soil, the complete root system has been recognised to enmesh soil particles resulting in the stabilisation of soil profiles (Kleinfelder *et al*., 1992) and improvement of soil conditions (Wheaton, McKenzie and Tisdall, 2008).

In addition to their physical activity, roots also influence the moisture content of the soil, causing wet/dry cycles to take place within the rhizospheric soil. As soil dries, cracks can be formed resulting in the failure of aggregates, whereas rapid wetting (e.g. after a rainfall event), can induce micro-cracks resulting in a more friable soil (Angers and Caron, 1998). Such chemical and physical impacts of roots on the soil structure can result in alterations in water flow paths (Hall *et al*., 1977; Noguchi *et al*., 1997) in addition to gas diffusion (Nye and Tinker, 1977) and the ease of microorganism movement.

1.3.3 EFFECTS OF MYCORRHIZAL FUNGI ON SOIL STRUCTURE

AMF influence soil structure through three different mechanisms (Rillig and Mummey, 2006). AMF within soil can, at the larger scale, influence the composition of plant communities present within the soil environment and lead to indirect effects which will ultimately influence soil structure. At the host level, AMF can influence soil structure in a number of ways, mostly through physical interaction. Finally, and most directly, AMF mycelium can have significant impacts on soil structure through biochemical, biophysical and biological interactions, all which will be discussed below.

1.3.3.1 Biochemical effects of mycorrhizal fungi

Firstly and most importantly are the direct effects fungal mycelia exert on soil structure at the hyphal scale within the soil environment. Fungal mycelia secrete fungal products such as glomalin, glomalin related soil protein (GRSP), mucilages, polysaccharides, hydrophobins and other compounds which influence soil structure

and in particular, soil aggregates. Since GRSP was first identified by Wright and Upadhyaya (1996) research into this protein has intensified and it has frequently been associated with the stabilisation of soil aggregates (Wright and Upadhyaya, 1998; Rillig, Wright and Eviner, 2002). Even with this increasing interest in the correlation between GRSP and soil aggregates, little research to date has assessed the true mechanism by which GRSP increases the water stability of aggregates. On the other hand, investigations into the presence of glomalin in soil have shown that it has the strongest influence on soil aggregate stability, in comparison with the direct effects that AMF hyphae have themselves (Rillig, Wright and Eviner, 2002).

In addition to glomalin, mucilages and polysaccharides from fungi are believed to influence soil structure (Chenu, 1989), however no study has yet investigated the release of these chemicals from AMF species. Similarly research into hydrophobins is somewhat limited. Hydrophobins (released from AMF) are believed to alter the polarity of the soil surface, altering the biotic and abiotic properties and thus could affect soil aggregation. Previous research suggests there is a close link with fungal-produced hydrophobins and the alteration of soil properties, even though published research surrounding this topic is limited and inconsistent (York and Canaway, 2000; Feeney *et al.,* 2006a, b).

Fungal mycelia also have direct influences on the microbiota and food webs within the soil environment. Since AMF species interact with other organisms present within the rhizosphere and the chemical composition of the soil, this can cause significant changes to the soil environment and thus in turn to other soil microbes. Mycelia products for example, which act as substrates for some microbiota, can lead to

changes in the bacterial communities (Filion, St-Arnaud and Fortin, 1999). In addition, AMF deposition products may also lead to alterations in microbial communities; which in turn may influence soil structure (Marschner and Baunmann, 2003). Furthermore, fungi form an important energy channel within soil food webs, which are vital for micro-arthropods and other soil meso-fauna. Since micro-arthropods have important direct roles in organic matter processing and thus indirectly affect soil structure, the effect AMF have on micro-arthropods could be extremely important (Rillig and Mummey, 2006) and *vice versa* (Klironomos and Ursic, 1998).

1.3.3.2 Biophysical effects of mycorrhizal fungi

Fungal mycelia can exert direct effects on the soil structure at the individual host root level. Firstly, the movement of the fungal mycelium itself can lead to important changes, resulting in the formation of macro-aggregates, as suggested by Tisdall and Oades (1982). This theory is supported by Bearden and Petersen (2000) where the strongest direct effect on the percentage of macro-aggregates within their study was caused by external hyphae. Hyphae act by enmeshing and entangling soil particles as well as producing a source of organic residues that create and support larger microbial populations (Tisdall and Oades, 1982; Miller and Jastrow, 1990). Besides supporting larger microbial communities, enmeshment of particles and organic matter aids the formation of larger structures such as macro-aggregates. The degree to which this enmeshment takes place will vary depending on the species of AMF present within the soil (Abbott and Robson, 1985). Hyphal morphology, including width, wall thickness and branching characteristics will vary with AMF species (Rillig and Mummey, 2006) causing variability in the tensile strength applied within the soil.

This in turn could directly influence soil aggregation, a hypothesis that has had little research to date.

In addition to macro-aggregation, the quantity and distribution of pore space within the soil environment may become altered. This may consequentially change the microhabitat, thus altering the microbial communities. In addition to enmeshment, hyphae influence particle alignment particularly that of clay particles which can be re-aligned within the soil. This can aid the binding of these clay particles to organic matter, which is vital for micro-aggregate formation, and further to that, macro-aggregate formation. Rillig and Mummey (2006) also suggest that hyphae within the rhizosphere influence wet-dry cycles within this environment, leading to the formation and destruction of both micro- and macro-aggregates.

1.3.3.3 The effect of mycorrhizal fungi on biological interactions and soil structure

When assessing the impact of AMF on soil structure it is vital to consider varying scales, including the plant level. AMF species composition within the soil influences plant communities and *vice versa*. Changes in plant communities will lead to changes in soil structure, as different plants have differing root architecture. Johnson *et al. (*2003) highlighted that AMF diversity within soil was significantly influenced by plant species composition. Differences in plant species may lead to different exudates and mucilages being released into the rhizosphere soil, altering microbial communities in response to chemical changes. This is supported by Rillig, Wright and Eviner (2002) who suggested that different plant species have different root lengths,

AMF hyphal length and glomalin concentrations and thus different effects on soil aggregate stability. Contrary to this, AMF diversity within the soil influences the productivity of plant communities (van der Heijden *et al.*, 1998b) and pathogen protection of plant roots (Sikes, Cottenie and Klironomos, 2009). Since plant productivity controls how much carbon eventually enters the soil, such factors are important for soil structure and in particular soil aggregation.

1.4 EFFECTS OF SOIL STRUCTURE ON MICROBES AND ROOTS

Microbial diversity plays an important part in determining the stability of soils, as well as ecosystem processes. These include residue decomposition, carbon sequestration, nitrogen fixing, organic matter/nutrient distribution, nutrient cycling, bioturbation, soil aggregation and population control. As described in section 1.3.1 and 1.3.3, microorganisms play an important role in creating and retaining soil structure (Young, 1998). In this dynamic interaction between soil microorganisms and soil structure, microbial communities are influenced by soil structure.

1.4.1 INFLUENCE OF SOIL STRUCTURE ON MICROBES

Pore size and connectivity within soil will influence microbial activity in addition to the dimensions of pore entrances, known as pore throats. Small pore throats can protect bacteria from predation, as potential pathways for other larger organisms e.g. protozoa, will be restricted. Heijnen, Hok-A Hin and van Veen (1991) demonstrated greater survival of *Rhizobium* in pores with a neck size of 6 μm. This effect of pore size on predation is mainly controlled by texture, with less predation in fine textured

soil than sandy soils (Rutherford and Juma, 1992). In addition to micro-pores acting as sites with lower predation rates, water immobilisation takes places within these micro-pores, restricting water and nutrient flow. This makes the environment favourable to bacteria since they are protected from extreme wet-drying cycles and desiccation that can influence microbial C and N dynamics and to some extent microbial community composition (Fierer and Schimel, 2002; Fierer, Schimel and Holden, 2003) and the input of toxic substances (Nishio and Furusaka, 1970; Hattori and Hattori, 1976; Foster, 1988; Ranjard *et al*., 1997).

The diffusion of gases, like water movement, also depends on the porous network within soil. Micro-pores have slow gaseous diffusion making the micro-pore environments anaerobic for some periods of time, resulting in the presence of anaerobic bacteria, like nitrifiers, colonising these areas (Philippot *et al.,* 1996). Other factors such as substrate availability within pore space will also determine the location of organisms within the soil. Organic matter within the soil is one of the main sources of carbon for heterotrophic microorganisms and the presence of small pores act as barriers to organic matter, thus limiting C-degradation, resulting in as much as 50-80 % of soil organic matter being located within these micro-aggregates (Christensen, 1992). The importance of C protection in smaller aggregates means that within larger aggregates microbial diversity is higher as microbial biomass and mineralisable C is higher (Lupwayi *et al*., 2001). In addition to acting as a substrate source, high organic matter content within these micro-aggregates modify the water retention, hydration and functioning of soil microorganisms by reducing the likelihood of desiccation (Chenu, 1993). Visualisation of the interactions between soil structure and soil microorganisms can be made using biological thin sections (section

1.1.3.3), however despite this, in order to increase our understanding of the impact soil structure has on microbial distributions within the soil (in addition to the various functions that take place within this complex environment), further development of modelling tools need to be developed (Young and Crawford, 2004).

1.4.2 INFLUENCES OF SOIL STRUCTURE ON ROOTS

Soil bulk density will influence root development as it passes through the soil. A highly compacted soil, with bulk density exceeding 1.55-1.85 Mg m^{-3} for example, depending on the soil type in question, will severely impede root development (Bowen, 1981) unless biopores are available which roots can utilise to reach water and nutrient stores (Stirzaker, Passioura and Wilms, 1996). Thus within the agricultural context the avoidance of soil compaction is paramount, particularly as very hard soils will prevent uptake of water and nutrients to the plant. The opposite should also be noted; loose structured soil reduces root-soil contact, resulting in a poor transport of water and nutrients (Veen *et al*., 1992; Atkinson, Sparkes and Mooney, 2009). The porosity of the soil and in particular pore space diameter is also vital in controlling roots. Roots tend to utilise old root channels and earthworm burrows in order to spread out within a soil, however if pore diameters are smaller than the root diameters, roots can experience difficulty in penetrating the soil (Wiersum, 1957). However Bengough, Croser and Pritchard (1997) demonstrated that root penetration does still take place in rigid pores smaller than a root's diameter, with latter suggestions linking lateral roots to penetration of these small pores (Clark, Whalley and Barraclough, 2003). Therefore roots may expand radially (due to ethylene release (Clark, Whalley and Barraclough 2003)), since elongational growth

is inhibited, causing the deformation of the soil surrounding the root and at the root tip making penetration possible (Hettiaratchi, 1990; Bengough and MacKenzie, 1994).

Factors, such as aeration, water and nutrient availability that are directly related to the porous network within soil, influence root activity in the soil environment. A good soil structure, consists of a range of pore sizes within and between aggregates, whose networks will control water, nutrient and gaseous movement. Root elongation is sensitive to limited soil aeration, with root elongation slowing and even stopping (Waters *et al*., 1991), particularly the primary lateral roots, which cannot develop effective adaptations in order to adjust to long-term anaerobic conditions (Laan, Clement and Blom, 1991). Younger plants and root systems however are able to adapt (Klaring and Zude, 2009). Under such anoxic conditions sugar transport from the shoots to the roots is inhibited by up to 79-97 % (Waters *et al*., 1991), nutrient accumulation is reduced, resulting in the reduction of plant growth associated with anoxia (Trought and Drew, 1980). Further effects of oxygen deficiency on soils have been described in the reviews by Drew (1997) and Drew and Lynch (1980). Anoxia within a 15 hour period has been recognised to cause irreversible damage to mitochondrial structure, energy metabolism and cell viability (Andreev, Generozova and Vartapetian, 1991). Furthermore with anaerobic conditions, the accumulation of reduced substances from anaerobic respiration e.g. NO_2^-, Mn_2^+, Fe_3^+ and H_2S, in addition to that of intermediate products of these processes, can result in reduced plant growth and even death due to phytotoxicity (Drew and Lynch, 1980). Such accumulation of phytotoxic substance also takes place during water-logging of the soil, where anaerobic conditions are also experienced due to reduced gaseous transfer between the atmosphere and the soil. Visser *et al. (*1997) also highlighted that soil

waterlogging led to the increase in ethylene gas in roots; deemed as having a stronger negative effect on root elongation than anoxia, whereas more recent work by Horchani *et al. (*2008) suggested that tomato quality was influenced more by disturbed growth regulators and increased ammonium due to water-logging than that of ethylene concentrations. Such work highlights that the degree of aeration within the soil is strongly related to the drainage ability of soil. The optimal soil structure conditions for roots are free draining, aerobic and with a high available water capacity, which also allow microorganisms to be involved in biochemical cycles to improve availability of nutrients such as nitrogen, phosphorus and calcium.

1.5 AIMS AND OBJECTIVES

The overall aim of the project is to examine the relationship between soil microbial diversity and soil structure in micro- and macrocosm systems of varying complexity. It is widely acknowledged that microbial activity plays an extremely important role in various soil processes, however what is not known is how biologically diverse a soil needs to be in order to develop and maintain its structure. With the current knowledge suggesting that climate change and human activity are causing changes in microbial communities due to species extinctions, the effect of changes in biodiversity have not yet been investigated in terms of the implications this may have on soil structure.

To address this aim, one key question will be asked:

What is the extent to which species extinctions affect the functioning of soil, particularly in terms of the stabilising effect on soil structure?

To answer this key question the following hypotheses were developed and tested throughout a series of investigations:

1. Pore size is the most important soil property for controlling microbial populations.

2. Soil structure (measured through total porosity, mean pore size, porous architecture, aggregate size and stability) will develop more rapidly when bulk soil microbial diversity is relatively high.

3. Presence of mycorrhizal fungi will enhance development of soil structure.

4. Combinations of arbuscular mycorrhizal fungal species will improve soil structure more rapidly than individual species.

1.6 THESIS OVERVIEW

An outline of the different experimental chapters within this thesis are presented in Table 1.3 where each individual experimental chapter are listed with the various methodologies used; these are described in the materials and methods chapter (Chapter 2). Chapter 6 goes on to discuss the overall findings from these experimental chapters, before the conclusions and implications of this work are highlighted in Chapter 7.

Table 1.3: Outline of experimental chapters within thesis.

CHAPTER NUMBER	EXPERIMENT TITLE	HYPOTHESES TESTED	METHODS USED
3	The effect of microorganisms on soil structural development	1,2	Microbial analysis • Microbial community metabolic analysis • Plate counts Structural analysis • X-ray µCT • Soil thin sections (using biological stains)
4	The effect of arbuscular mycorrhizal fungi and roots on the development of soil structure.	1,2,3	Plant analyses • Shoot biomass • Root biomass Soil analysis • Organic Matter Microbial analyses • Microbial community metabolic analysis • Soil Biomass • Mycorrhizal colonisation • T-RFLP Structural analyses • X-ray CT Scanning • Aggregate size distribution • Aggregate stability • Aggregate water repellency
5	Impact of mycorrhizal fungi on soil structure development.	2,3,4	Plant analyses • Shoot biomass • Root biomass Soil analysis • Organic Matter Microbial analyses • Soil Biomass • Mycorrhizal colonisation • Hyphal penetration • T-RFLP Structural analyses • X-ray CT Scanning • Aggregate size distribution • Aggregate stability

2 GENERAL METHODS

2.1 COLLECTION OF SOIL AND GENERAL HARVEST TECHNIQUES

2.1.1 SOIL COLLECTION AND PROCESSING

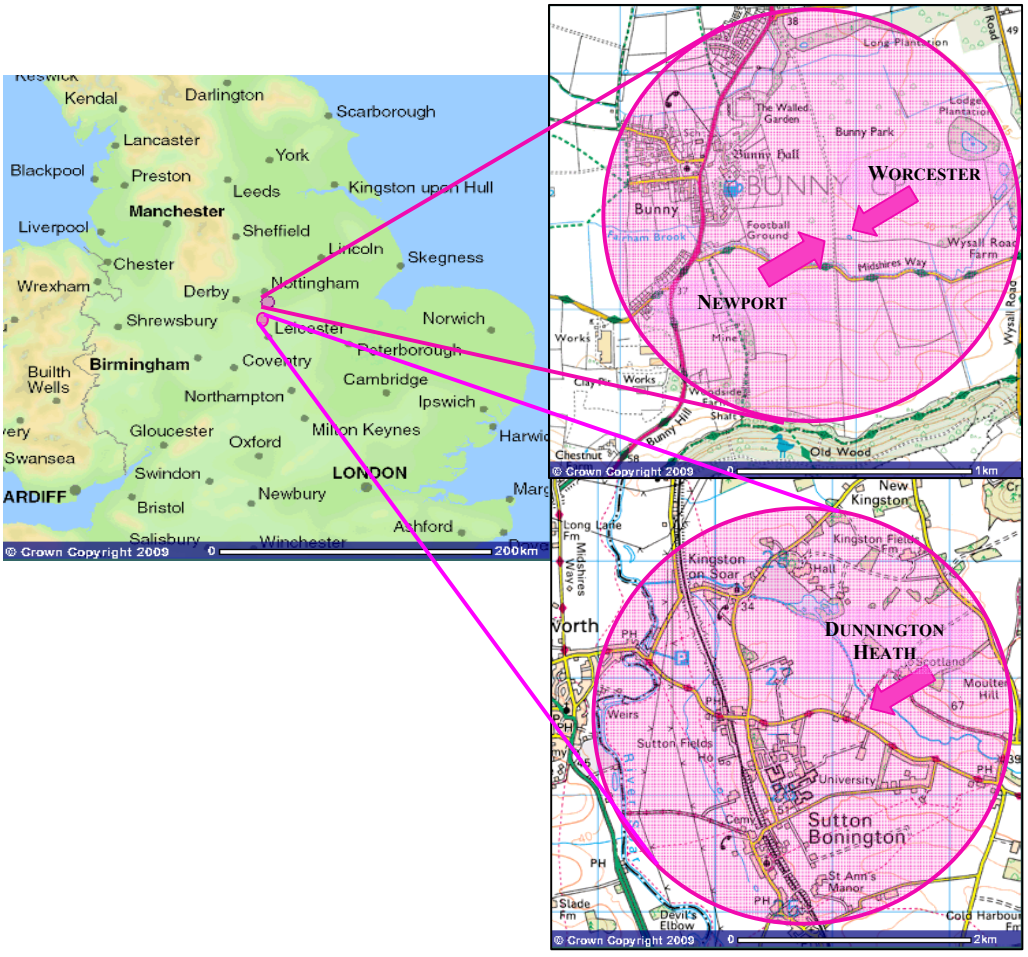


Figure 2.1: Site location where the loamy sand (Newport series), clay loam (Worcester series) and sandy loam (Dunnington heath series) were collected.

In all experiments top soil (5-20 cm depth) was either collected from one or three different field sites each with different soil textures. The soils examined included the Newport series, a loamy sand (brown sand) and Worcester series, a clay loam

(Argillic Pelosol) from the University of Nottingham's experimental farm site at Bunny, Nottinghamshire (GB Ordnance Survey Grid Reference: SK 587 294 and SK 587 289 respectively) and the Dunnington Heath series, a sandy loam (Stagno Glegic Luvisol) from the University of Nottingham farm site at Sutton Bonington, Leicestershire (GB Ordnance Survey Grid Reference: SK 512 267) (Figure 2.1). This Dunnington Heath (sandy loam) top soil was used within all experiments. Selected soil physical and chemical characteristics of these soils are given in Table 2.1 and Figure 2.2.

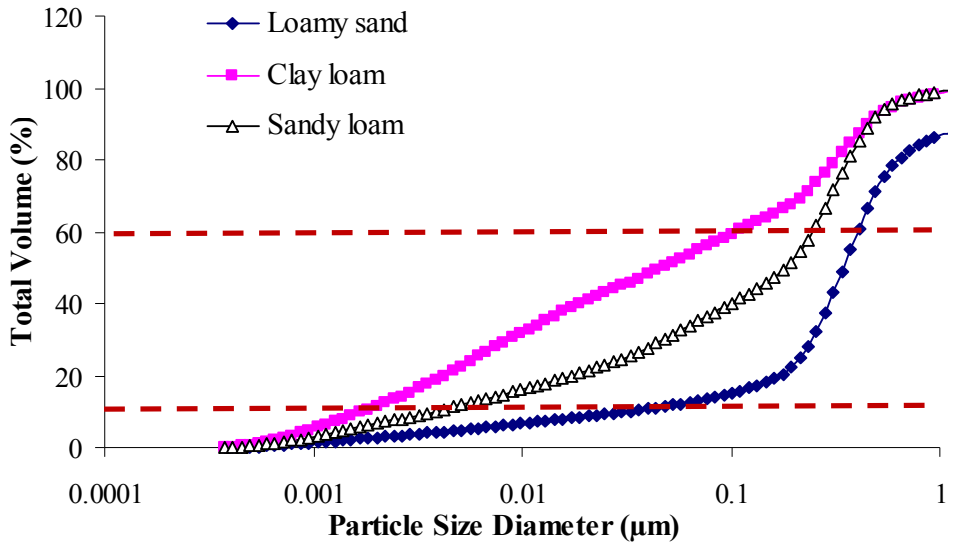


Figure 2.2: Particle size distribution of each of the three soils used within the experiment (determined by laser particle analysis).
Note: Red lines indicate the 10 % and 60 % points at which the coefficient of uniformity is calculated (section 2.2.3).

Table 2.1: Characteristics of soils used in microcosms

Particle size analysis of the samples was undertaken using laser particle analysis (section 2.2.1).

Soil Series	Soil Type	Sand (%)	Silt (%)	Clay (%)	Organic Matter (%) *	pH
Worcester	Clay Loam	31.1	34.5	34.4	5.19	6.50
Newport	Loamy Sand	78.7	9.4	11.9	2.98	7.06
Dunnington Heath	Sandy Loam	66.4	18.0	15.6	3.73	7.35

* Organic matter content determined by loss on ignition.

Upon collection, the soil was air dried and sieved to < 2 mm before sealing the processed soil in double plastic bags containing ~ 7-8 kg for sterilisation using gamma radiation (Isotron Ltd. Daventry, UK) unless otherwise stated.

2.1.2 INOCULATION OF SOIL

After soil processing and sterilisation, soil was packed into microcosms and macrocosms of varying sizes, as stated in the appropriate experimental chapters. Experimental macrocosms were inoculated using the dilution technique (Salonius, 1981; Griffiths *et al.,* 2001). Soil micro- or macrocosms were inoculated using a soil slurry solution, made from fresh field soil (taken from the respective field site where the soil texture was collected from) by diluting it in ¼ strength sterile Ringers solution (where full strength Ringer solution is: 2.25 g NaCl, 0.105 g KCl, 0.12 g $CaCl_2$ and 0.05 g $NaHCO_3$ dissolved in 1 L of sterile de-ionised water (Dickinson Austin and Goodfellow, 1975)). The soil slurry solution was made to differing dilutions depending on the experiments in question. A 10^{-1} soil suspension was prepared by mixing 100 g of field fresh to 1000 ml ¼ strength sterile Ringers solution, with

subsequent serial dilutions make to a 10^{-6} dilution. Generally however either a 10^{1} (low), 10^{6} (high) or no dilution (i.e. just sterilised water) was used to inoculate experimental soil.

Inoculation involved saturating the sterilised air-dried micro- or macrocosms overnight in a specifically diluted soil slurry solution (by placing the micro- or macrocosms in trays containing the inocula) allowing capillary uptake of the solution containing microorganisms throughout the soil. Once the cores were saturated, they were removed from the solution and left to drain for 2 days to reach field capacity and weighed prior to the start of experiments.

Depending on the experimental setup, some macrocosms also underwent planting with *Plantago lanceolata* (Herbiseed, Twyford, UK) either by transplanting seedlings or by growing *P. lanceolata* directly from seeds within the macrocosms. *P. lanceolata* was selected for the experiment due to its known mycotrophy (Šmilauer, 2001) and since AMF colonisation does not affect the lifespan of *P. lanceolata* roots (Hodge, Robinson and Fitter, 2000).

2.1.3 HARVEST TECHNIQUES

Soil macrocosms were destructively harvested at specific harvest periods after inoculation and plant transplanting or establishment for microbial and structural assessment. At each harvest above ground plant biomass (from treatments containing plants) was determined by removing the plant at the soil level before macrocosm destruction. Soil from each sampled macrocosm was removed gently to prevent destruction of the soil aggregates and damage to roots (where present). All possible

root material was gently removed from the soil, with subsections removed and stored in 70 % ethanol or frozen at -80 ^{o}C for assessment of AMF colonisation, with the remaining root material used to estimate the total below ground plant biomass (after taking into account the weight of the undried subsample). Soil removed from macrocosms was homogenised gently prior to sub-sampling for immediate determination of soil moisture, organic matter content (loss on ignition) and metabolic potential. Additional subsamples were taken for soil biomass and relative abundance determination and stored at -20 ^{o}C and -80 ^{o}C respectively. A subsample of soil, for assessment of the soil structure i.e. (aggregate size distribution, aggregate stability and water repellency), was also removed and left to air dry.

Additional soil structural analysis was undertaken using X-ray μCT and X-ray CT depending on column size. Separate micro- and macrocosms were specifically used for this assessment for each experiment. In all experiments the same set of macrocosms were scanned to allow changes in soil structure overtime to be assessed.

2.2 Analysis of Soil Physical Properties

This section focuses on the techniques used to determine soil texture, soil aggregate stability, total porosity and pore size and morphology.

2.2.1 Soil Texture

Air dried soil was sieved to < 2 mm in size, with 0.5 g weighed into a 50 ml centrifuge tube. Soil organic matter was chemically removed from the soil using 25 ml of hydrogen peroxide (H_2O_2) overnight. To ensure all organic matter had been

removed from the soil sample, the centrifuge tube was placed in a 60 ^{o}C water bath for 1-1.5 hours with the temperature raised to 90 ^{o}C for an additional 1-1.5 hours. Samples were topped up with 25 ml of deionised water prior to centrifuging at 3500 rpm for four minutes. The remaining solution was decanted off, with an additional 35 ml deionised water added to the sample prior to centrifuging at 3500 rpm for four minutes again. The remaining solution was decanted and 25 ml of calgon (35 g of sodium hexametaphosphate, 7 g sodium carbonate in 1 L of de-ionised water) added before shaking and placing in an ultrasonic bath for 30 minutes. Samples were then analysed in a particle size analyser (Beckman Coulter LS230, Beckman Coulter Inc., High Wycombe, UK).

2.2.2 SOIL MOISTURE AND ORGANIC MATTER CONTENT

Soil moisture at column harvest and organic matter content were determined by oven drying samples and determining the loss on ignition (Rowell, 1994).

Soil samples from the soil columns were placed in weighed crucibles. The water content of soils was determined by drying at 105 ^{o}C overnight and using Equation 2.1.

$$\text{Water content} = \frac{\text{Mass of fresh soil} - \text{Mass of oven-dry soil}}{\text{Mass of oven-dry soil}} \quad \text{(Eq. 2.1)}$$

Equation 2.1: Determination of soil water content.

The oven dried soil was then heated to 500 ^{o}C for 8 hours. After the crucibles had cooled, they were re-weighed to give the mass of ignited soil. The mass lost by ignition was determined using Equation 2.2.

$$\text{Loss on ignition} = 100 \times \frac{\text{Mass of oven-dry soil} - \text{Mass of ignited soil}}{\text{Mass of oven dry soil}} \quad \text{(Eq. 2.2)}$$

Equation 2.2: Determination of loss of ignition.

2.2.3 AGGREGATE SIZE DISTRIBUTION

Soil removed from each experimental soil column was air dried for 7-14 days. After drying, 25 g was taken from the dried homogenised soil subsample and gently sieved by hand through nine sieves: 4000, 2000, 1000, 500, 425, 300, 212, 106 and 53 µm. The mass retained on each sieve was weighed, recorded and the percentage mass in each fraction calculated. From aggregate size distributions (Figure 2.2), the coefficient of uniformity (Kézdi, 1974) (also termed the Hazen coefficient) was used to numerically illustrate the differences in distributions where large and small aggregates co-existed (Equation 2.3). This allows the ratio of aggregates at 10 % and 60 % of the aggregate size distribution to be determined; the larger the ratio, the greater the number of larger aggregates and greater uniformity in the distribution of these aggregates (Figure 2.2; Table 2.2).

$$ASD_{CU} = \frac{d_{60}}{d_{10}} \quad \text{(Eq. 2.3)}$$

Equation 2.3: Coefficient of uniformity for aggregate size distribution (ASD).
Where: d_{10} = size of aggregates at 10 % of the total soil volume and d_{60} = size of aggregates at 60 % of the total soil volume

Table 2.2: Boundaries for the coefficient of uniformity for aggregate size distributions.

Class Boundaries	Ratio value
Very Uniform (more larger aggregates)	< 5
Medium Uniform	5
Not Uniform (more smaller aggregates)	>5

2.2.4 AGGREGATE STABILITY

The fast wetting (slaking) technique, developed by Le Bissonnais (1996) was used as it is preferable to other published techniques because it is simple, rapid and a quantitative test for aggregate stability.

Soil removed from experimental columns, was air dried for 7-14 days and sieved to 2-5 mm in size. These 2-5 mm aggregates were oven dried at 40 ^{o}C for 24 hours, and 5 g removed for analysis. Aggregates were gently immersed into a 250 ml beaker filled with 50 ml of de-ionised water for 10 minutes (Figure 2.3). After this the water was carefully siphoned off with a pipette, the soil material transferred to a 53 μm sieve and then immersed in ethanol to avoid re-aggregation and restrict further breakdown of soil aggregates. The 53 μm sieve was gently agitated with great care to avoid further breakdown of aggregates but to allow separation of the >53 μm fraction. The sieve containing the remaining soil was placed in an oven overnight at 105 ^{o}C to dry.

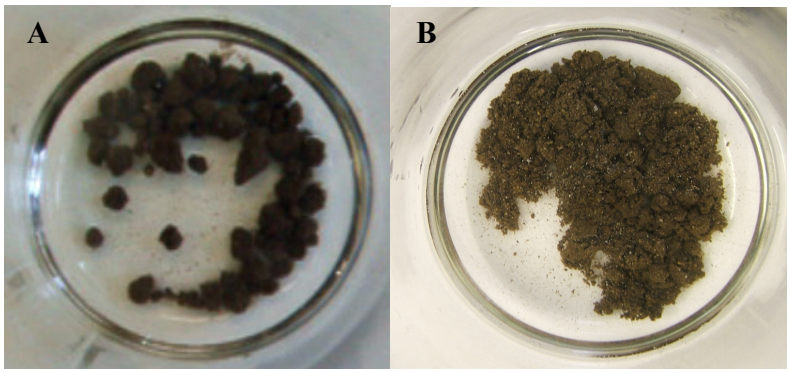


Figure 2.3: Aggregate stability slaking test. A) Shows aggregates initially placed in water, B) aggregates after submersion for 10 minutes.

After drying, the soil material was gently sieved through six sieves: 2000, 1000, 500, 200, 100 and 53 μm. The mass retained on each sieve was weighed, recorded and the percentage mass in each fraction calculated. The fraction < 53 μm was calculated from the difference between the initial mass and the sum of the six other fractions. The aggregate stability measured by this breakdown mechanism is expressed either as the fragment size distribution (FSD) in seven classes or the mean weight diameter (MWD) which is the sum of the mass fraction remaining multiplied by mean aperture of adjacent mesh. The calculation of MWD can be expressed in mathematical terms (Equation 2.4; Van Bavel, 1949; Kemper and Rosenau, 1986). The boundaries of MWD, indicating the degree of aggregate stability are shown in Table 2.3.

$$\text{MWD} = \sum (X_i W_i) \quad \text{(Eq.2.4)}$$

Equation 2.4: Mean weight diameter (MWD).
Where x_i is the average diameter of openings of two consecutive sieves and W_i is the weight ratio of aggregates remaining on the i^{th} sieve.

Table 2.3: Boundaries of soil aggregate stability according to mean weight diameter.

Class Boundaries	MWD (mm)
Very Unstable	< 0.4
Unstable	0.4 - 0.8
Medium	0.8 - 1.3
Stable	1.3 - 2.0
Very Stable	> 2.0

2.2.5 PORE MORPHOLOGY ASSESSMENT USING X-RAY COMPUTED TOMOGRAPHY

Prior to destructive sampling, all soil columns were scanned non-destructively using a Venlo H series, high resolution X-ray CT Scanner (H 350/225 CT; X-TEK, Tring, Hertfordshire, UK) (Figure 2.4) unless otherwise stated. The exact scanning protocol, including power levels and scanning times, varied with each experiment but the following was common throughout. A 2 mm primary copper filter was placed near the X-ray source to eliminate X-ray scatter, in addition to a 4 mm secondary copper filter placed at the detector to prevent detector saturation (i.e. when the input to the detector exceeds the total capacity) and beam hardening (Figure 2.5 and 2.6). Beam hardening is an artefact created when the average energy of an X-ray beam increases as the beam propagates through a material as the low energy X-rays are attenuated preferentially. Such incidences must be prevented since beam hardening and saturation can compromise any image analysis (Figure 2.7).

The detector used for all scans consisted of 3710 diodes set 83 μm apart. Gain and offset correction was applied to all of the diodes within the detector by applying a black (offset) and white (gain) reference to adjust for exposure variations. Each

sample was scanned at pre-determined depths according to each particular experimental layout.

Figure 2.4: Example of Venlo H CT scanner used during investigations.

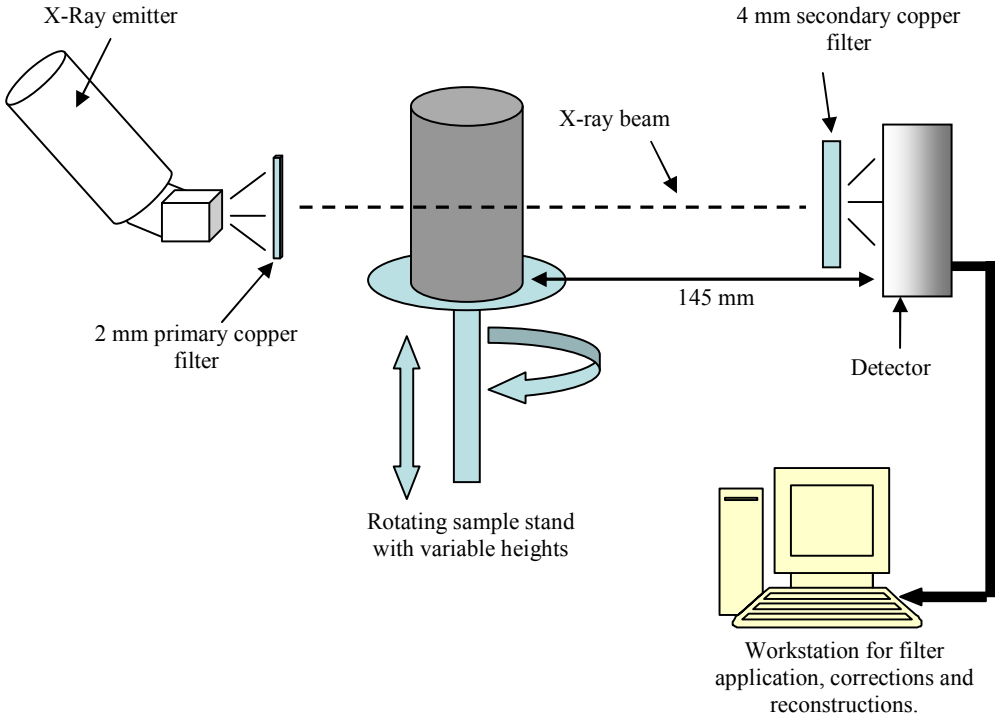


Figure 2.5: X-ray Computed Tomography system.

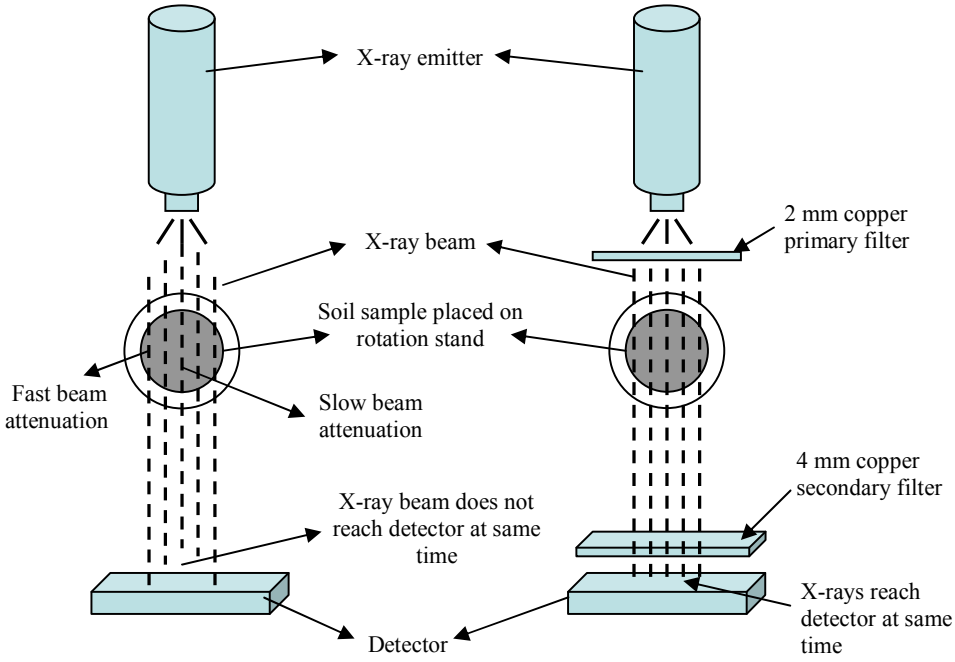


Figure 2.6: Beam hardening correction using primary and secondary copper filters.

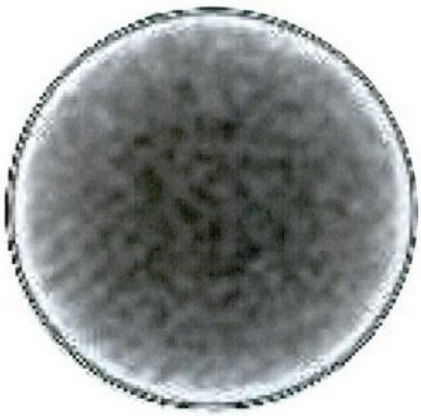


Figure 2.7: Example of beam hardening in a CT image of a sand filled column (taken from Akin and Kovscek, 2003).
Note: The lighter shading that occurs just inside the column that represents effects of beam hardening.

Images obtained from X-ray CT were processed in order to reduce background noise introduced into the image by the scanning and reconstruction process. The image analysis technique applied to each image set varied according to the machine used and

noise present within each scanning period, with the image analysis techniques described according to their application within each respective experiment. From processed binary images measurements regarding the pore size, distribution and morphology (i.e. shape) were determined. Total porosity of the image was determined as a percentage of pores within the total sampling area. Mean pore size was a mean value of the size of each pore present within the sampled area; with a pore size distribution (PSD) also determined for each image, displaying the range of pore sizes present within the sample. A logarithmic scale to display pore size classes was devised to best separate pore sizes throughout the different micro- and macrocosms (Table 2.4). Furthermore the closeness of pores to each other is determined by calculating the nearest neighbour distance. Additional measurements of a pore's perimeter can also be made to assess the roughness of a pore's surface (Atkinson, 2008). Given that pore perimeter follows a close and significant relationship with mean pore area (Kampichler and Hauser, 1993; Pachepsky *et al.,* 1996), only selected perimeter data is presented here. Morphological pore measurements included pore circularity (sphericity), a value given between zero and one to signify how circular a pore is; a value of 0 indicates an elongated pore and a value of one indicates a circular pore. Circularity of pores is estimated by dividing the product of area of the pore and 4π by the pore perimeter squared (Equation 2.5) (Tuller, Or and Dudley, 1999) and is sometimes referred to a pore's shape factor (F) whose value determines the shape of a pore (Bouma *et al*., 1997)

$$C = \frac{A \times 4\pi}{p^2} \quad \text{(Eq. 2.5)}$$

Equation 2.5: Pore circularity (Tuller, Or and Dudley, 1999).
Where: C = pore circularity; A= pore area; p = pore perimeter.

Table 2.4: Equivalent size of pores (mm^2) of logarithmic size classes used in pore size distribution results.

Size class (log)	Equivalent pore size (mm^2)
-3	0.001
-2.5	0.00316
-2	0.01
-1.5	0.0316
-1	0.1
-0.5	0.316
0	1
0.5	3.16
1	10
1.5	31.6
2	100
2.5	316
3	1000

2.3 SOIL MICROBIAL MEASUREMENTS

The following methods were chosen to provide data relating to the microbial community structure, metabolic potential and biomass within the experimental soils.

2.3.1 METABOLIC POTENTIAL OF THE SOIL MICROBIAL COMMUNITY

The metabolic potential of the heterotrophic soil microbial community was determined by Biolog® microtitre plates (Garland and Mills, 1991; Zak *et al*, 1994). Fresh soil samples were manually homogenised within the sample bag. A soil dilution of 10^2 was made by suspending 1 g of dry weight equivalent soil in 100 ml of ¼ strength Ringer's solution. The suspension was mixed thoroughly prior to inoculation by hand shaking for 10 seconds and briefly vortexing.

Biolog plates (Biolog Inc., California, USA; supplied by Techno-path Distribution Ltd, Limerick, Ireland) were inoculated with 120 µl per well of 10^2 soil suspension and incubated at room temperature (or 20 ^oC) for 5 days. Colour intensity within each

well was determined using a microplate reader (BioTek ELX808, BioTek Instruments, Inc., Vermont, USA) within 2 hours of inoculation (to allow removal of background absorbance introduced from inoculation) and also at 24, 48, 72, 96 and 120 hours incubation. Colour intensity was assessed by the analysis software Gen5 (BioTek Instruments, Inc., Vermont, USA), before data manipulation within Excel (Microsoft® Excel 2003) to correct for background and inoculation induced absorbance (determined from colour intensity values 2 hours after inoculation). The 95 substrates within the microtitre wells were grouped into guilds according to the type of carbon substrates, e.g. carbohydrates, carboxylic acids, amino acids (see Table 2.5 for additional categorisation). The proportion of wells of each substrate type showing utilisation (or colour development) over a value of 0.25 was assessed (Garland, 1997) and the proportion of the total number of substrates that were utilised at each measured time point. In addition, the average well colour development (AWCD) was calculated for each plate and reading time as the mean colour intensity of the 95 carbon substrates which had colour development values greater than 0.25 (Garland and Mills, 1991; Garland, 1997). Garland (1996) recommended dividing each individual colour score by the AWCD for the plate to normalise the data set. A repeated measures analysis of variance (ANOVA) was performed on AWCD data at each incubation period with harvest and soil type as factors. Furthermore the rate of total substrate utilisation change was also determined between appropriate incubation times and statistically tested using an ANOVA. Principal component analysis (PCA) was used to identify patterns within data sets. AWCD data was transformed using a natural log transformation prior to PCA assessment. The covariance matrix was used, according to Glimm *et al. (*1997) as it does not sacrifice data within large multivariate

data sets such as the 95 colour intensity measurements for each sample plate. PC loading values were analysed using an ANOVA.

2.3.2 SOIL BIOMASS BY FUMIGATION-EXTRACTION

Soil microbial biomass C was determined in experimental soils using the method of Vance, Brookes and Jenkinson, (1987). Soil samples were defrosted for 48 hours (within a cold room) prior to fumigation-extraction. Two sets of triplicate samples from three replicate columns were weighed in (20 g dry weight equivalent of soil); one set of triplicate samples was fumigated with chloroform prior to extraction with potassium sulphate, whilst the second set were extracted immediately.

Table 2.5: Carbon Sources in Biolog GN2 microtitre plates.

Yellow = Polymers; Blue = Carbohydrates; Lime green = Carboxylic Acids; Pink = Amino Acids; Orange = Esters; Peach = Brominated chemicals; Purple = Amides; White = Aromatic chemicals; Red = Amines; Green = Alcohols and Grey = Phosphorylated Chemicals

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	α-Cyclo-dextrin	Dextrin	Glycogen	Tween 40	Tween 80	N-Acetyl-DS-Galactosamine	N-Acetyl-D-Glucosamine	Adonitol	L-Arabinose	D-Arabitol	D-Cellobiose
B	i-Erythritol	D-Fructose	L-Fucose	D-Galactose	Gentio-biose	α-D-Glucose	m-Inositol	α-D-Lactose	Lactulose	Maltose	D-Mannitol	D-Mannose
C	D-Melibiose	β-Methyl-D-Glucoside	D-Psicose	D-Raffinose	L-Rhamnose	D-Sorbitol	Sucrose	D-Trehalose	Turanose	Xylitol	Pyruvic Acid Methyl Ester	Succinic Acid Mono-Methyl-Ester
D	Acetic Acid	Cis-Aconitic Acid	Citric Acid	Formic Acid	D-Galactonic Acid Lactone	D-Galactu-ronic Acid	D-Gluconic Acid	D-Gluco-saminic Acid	D-Glucuronic Acid	α-Hydroxy-butyric Acid	β-Hydroxy-butyric Acid	γ-Hydroxy-butyric Acid
E	p-Hydroxy Phenylactic	Itaconic Acid	α-Keto Butyric Acid	α-Keto Glutaric Acid	α-Keto Valeric Acid	D,L-Lactic Acid	Malonic Acid	Propionic Acid	Quinic Acid	D-Saccharic Acid	Sebacic Acid	Succinic Acid
F	Bromosuc cinic Acid	Succinami c Acid	Glucurona mide	L-Alaninamid e	D-Alanine	L-Alanine	L-Alanyl-glycine	L-Asparagine	L-Aspartic Acid	L-Glutamic Acid	Glycyl-L-Aspartic	Glycy-L-Glutamic Acid
G	L-Histidine	Hydroxy-L-Proline	L-Leucine	L-Ornithine	L-phenylalani ne	L-Proline	L-Alayl-Glycine	D-Serine	L-Serine	L-Threonine	D,L-Carnitine	γ-Amino Butyric Acid
H	Urocanic Acid	Inosine	Uridine	Thymidine	Phenyethy lamine	Putrescine	L-Pyro-glutamic Acid	2,3-Butanediol	Glycerol	D,L-α-Glycerol Phosphate	α-D-Glucose-1-Phosphate	D-Glucose-6-Phosphate

2.3.2.1 Fumigation

Beakers (labelled in pencil) of soil were placed into a desiccator lined with moist tissue paper at the base, in addition to a vial of 25 ml of soda lime and a 50 ml beaker of at least 40 ml $CHCl_3$. The rim of the desiccator lid was covered with silicon gel, prior to evacuation of the desiccator to aid sealing. Desiccators were evacuated until the $CHCl_3$ boiled vigorously, or for 4 minutes so a firm seal was made. The desiccator was left at room temperature for 24 hours in the dark. After this period a vacuum should be present within the desiccator. If not and liquid $CHCl_3$ still remains in the beaker, (within the desiccators), fumigation may be considered successful.

After fumigation, the moist tissue paper, chloroform and soda lime were removed and the desiccator (including soil samples) evacuated for two minutes, three times to remove the smell of $CHCl_3$. The soil samples were transferred into 250 ml plastic screw-top bottles, with the glass beakers rinsed with K_2SO_4 to remove all soil particles, if necessary.

2.3.2.2 Extraction

To the fumigated and unfumigated soil samples, 0.5 M K_2SO_4 was added in a ratio of 4:1 (i.e. 20 g dry weight equivalent soil was extracted with 80 ml K_2SO_4). The bottles were placed upright on a reciprocal shaker set at 200 strokes min^{-1} and shaken for 1 hour, including three blanks of K_2SO_4. Once removed from the shaker, bottles were inverted to re-suspend the soil. The complete extract was filtered (Whatman, No. 42, 15 cm) into a polythene bottle. Filtered extracts were frozen at -20 ^oC until analysis.

Extracts were defrosted at 4 ^{o}C for 12 hours before carbon analysis. On thawing, a white precipitate of $CaSO_4$ usually appeared in the extracts. These bottles were shaken thoroughly and allowed to stand so the precipitate re-settled. Extracts were diluted 1: 10 ml before analysis using the Total Organic Carbon analyser (TOC – VCPH/CPN, Shimadzu Corporation, Koyoto, Japan). A calibration curve in the range 2 – 10 mg CL^{-1} was created using a total organic carbon standard solution of 1000 mg CL^{-1}. The standard was made by dissolving 2.125 g of potassium hydrogen phthalate, previous dried at 105-120 ^{o}C for 1 hour and cooled in a desiccator, in 1 L milli-q water.

Biomass (Bc) was calculated from Equation 2.6

$$B_C = \frac{E_C}{0.45} \qquad \text{(Eq. 2.6)}$$

Equation 2.6: Soil microbial biomass calculation (Jenkinson, Brookes and Powlson, 2004).

Where Ec = (C extracted from fumigated soil) minus (C extracted from non- fumigated soil) after the dilution factor is corrected for Wu et al. (1990).

2.3.3 TERMINAL-RESTRICTION FRAGMENT LENGTH POLYMORPHISM (T-RFLP)

Characterisation of the microbial communities present within the soil treatments was undertaken using Terminal-Restriction Fragment Length Polymorphism (T-RFLP). T-RFLP is a direct DNA profiling method using fluorescently labelled primers during PCR amplification. The digestion of the PCR labelled product with restriction enzymes, generates fragments of DNA of differing lengths which are related to different bacterial or fungal species present within a sample. The digested sample is then placed into a DNA sequencer system which detects the labelled primer and from this, records the size of the corresponding fragment and its relative abundance.

2.3.3.1 DNA extraction

DNA was extracted from the soil using a PowerSoil DNA kit (Mo-Bio Laboratories Inc., Carlsbad, California, USA) since this particular kit enables DNA cleaning. Briefly, 0.25 g of defrosted soil was added to a PowerBead Tube and vortexed. Solution A* was added to the tube prior to mixing on a flat bed vortex for 10 minutes to allow cell lysis, before being centrifuged at 11,731 rpm (10,000 x Gravity (*g*)) for 30 seconds. The supernatant was transferred into an Eppendorf tube before addition of solution B* to precipitate out the non-DNA components during incubation at 4 ^{o}C for 5 minutes prior to centrifuging at 11,731 rpm for 30 seconds. Avoiding the pellet, the supernatant was transferred into a new Eppendorf before addition of solution C* which precipitated the non-DNA components during a minute incubation period at 4 ^{o}C, before centrifuging again at 11,731 rpm for 30 seconds. Avoiding the pellet, the supernatant was transferred into a clean Eppendorf, where solution D* allowed the binding of DNA to the added spin filter, prior to vortexing.

The supernatant was then loaded onto a spin filter in stages, before centrifuging the spin filter at 11,731 rpm for 1 minute with the flow through discarded. After all the sample had passed through the spin filter, the DNA was washed and cleaned using solution E* and centrifuged at 11,731 rpm for 30 seconds. The spin filter was then placed into a clean Eppendorf, before a DNA elution solution F* was added, which removes the DNA from the spin filter into solution, while the sample was centrifuged at 11,731 rpm. The sample was frozen at -80 ^{o}C prior to PCR amplification.

* Identity of solutions A, B, C, D, E, and F was not disclosed by the manufacturer.

2.3.3.2 PCR for T-RFLP

DNA extracted from the soil was amplified in the ITS-2 region for fungi and the 23S ribosomal subunit for bacteria. The fungal primers amplify the ITS-2 region by priming from the 5.8S rRNA (5'-GCA TCG ATG AAG AAC GCA GC-3'). The fungal reverse primer (FITS rev) was labelled with a green dye D3 (5'-dyeD3 ATA TGC TTA AGT TCA GCG GGT-3') (Sigma-Genosys, Haverhill, Suffolk). The bacterial 23S primers amplify the 23S ribosomal subunit (Anthony, Brown and French, 2000) by priming with 23Sfor (5'-GCG ATT TCY GAA YGG GGR AAC CC-3') and the reverse primer (23Srev) was labelled with a blue dye D4 (5'-dyeD4 TTC GCC TTT CCC TCA CGG TAC T-3') (Sigma Proligo, Gillingham, Dorset).

One µl of the sample DNA was added to 24 µl of the PCR solution, prior to the PCR as described in Table 2.6. The PCR solution was made up using the specific reagent ratios; 12.5 µl of 2 x PCR Master mix (containing the following volume ratios 3µl 10 x PCR buffer, 2.4 µl 25 mM $MgCl_2$, 0.3 µl 25 nM dNTPs and 0.3 µl Taq DNA Polymerase; Promega, Southampton, UK), 11.5 µl of sterile de-ionised water, 0.5 µl of 20 pmol forward primer (i.e. fungal or bacterial) and 0.1 µl of 20 pmol reverse primer (i.e. fungal or bacterial). Note that each DNA sample was amplified using both the fungal and bacterial primer, but in individual tubes.

PCR products were tested for success on 1 % agarose gels in 1 x TBE buffer containing 1 % ethidium bromide (Sigma Aldrich, UK). Once the gel had set, 5 µl of PCR product was mixed with 5 µl of Orange G loading dye (Sigma Aldrich, UK) on Nescofilm. Eight µl of this PCR product and Orange G loading dye mix was loaded into each well

in the agarose gel (within the electrophoresis tank). In the end wells, one negative sample (i.e. the PCR mix plus sterile water instead of a DNA sample) and 5 µl of 1 KB DNA ladder were added. Once complete the gel was run at 120 V for 1 hour, to allow the bands to dissipate down the gel according to the fragment size. After an hour the gel was observed under a UV light and imaged (Figure 2.8). Samples which failed at the PCR stage were cleaned in a pre-moistened spinfilter containing polyvinylpolypyrrolidone (PVPP) (Sigma Aldrich, UK) and centrifuged for 2 minutes.

Table 2.6: PCR reaction settings.

TEMPERATURE AND TIME SETTINGS	PCR STAGE
94°C for 2 minutes	Initial denaturing.
94°C for 30 seconds	35 cycles
53°C for 1 minute	
72°C for 1.5 minutes	
72°C for 15 minutes	Final extension step
4°C Hold	Holding conditions prior to removal from PCR machine.

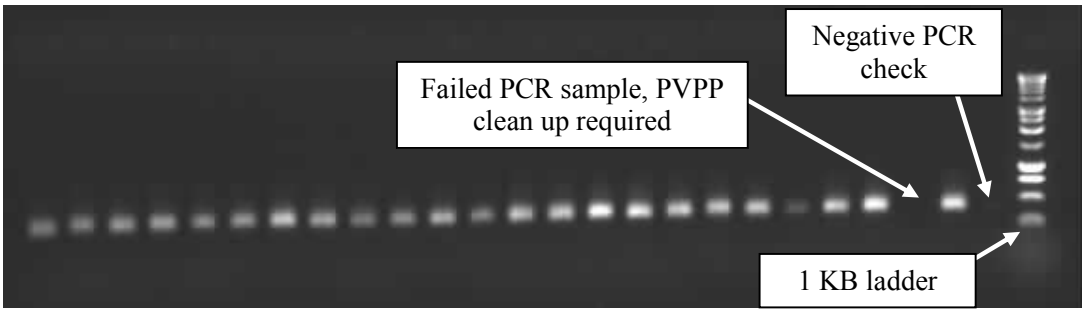


Figure 2.8: Example of electrophoresis gel for PCR samples.
Each white band represents a sample which has been successfully amplified. Any wells with a faint PCR sample, had the PCR repeated to produce a stronger DNA sample that would be suitable for restriction digestion. Wells containing no visible bands required PVPP clean up.

2.3.3.3 Restriction digest

After amplification of the DNA through PCR, the PCR product was digested prior to fragment analysis. Briefly, 9 µl of the restriction solution (made using the following reagents ratios; 7 µl of sterile de-ionised water, 2 µl of restriction enzyme buffer (buffer 2 (New England BioLabs, Hitchin, Hertfordshire, UK) for fungal, or buffer C (Promega, Southampton, UK) for bacterial samples) and 0.1 µl of restriction enzyme (*Hae*III for fungal samples (New England BioLabs, Hitchin, Hertfordshire, UK) or *Mse*I for bacterial samples (Promega, Southampton, UK))) to 10 µl of PCR product. The samples were placed in an incubator at 37 ^{o}C for 4 hours to allow digestion. Successful digests were separated on 1 % agarose gels in 1 x TBE buffer containing 1 % ethidium bromide and run at 120V for 1 hour (using method in section 2.2.3.2) (Figure 2.9).

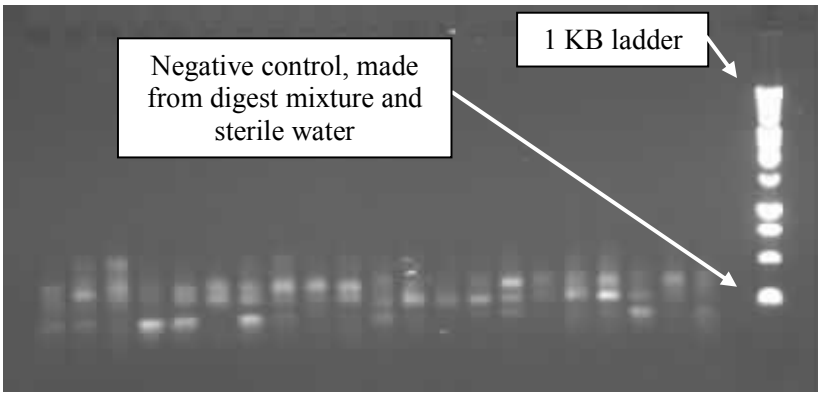


Figure 2.9: Example of electrophoresis gel for restriction digest samples.
Each lane on the gel represents a sample which has been successfully digested producing fragments of various length (hence the white fuzzy zone within each well). Any wells without visible products required re-digestion.

2.3.3.4 T-RFLP fragment analysis

In 0.5 ml Eppendorfs, 3 µl of the bacterial restriction digest was mixed into 3 µl of the respective fungal restriction digest and vortexed to mix. Both bacterial and fungal fragments for a single sample were loaded into the same well within the fragment analysis plate, since different dyes were used for each.

In each well of the fragment analysis plate, 39 µl of a master mix consisting of 320 µl of the sample loading buffer (Beckman Coulter Inc, High Wycombe, UK) and 4 µl of the internal 600 base pairs (bp) standard ladder (Beckman Coulter Inc.) was added. This ladder allows fragments between 60-640 bp to be considered during analysis. One µl aliquots of the mixed digest sample was loaded into each well and mixed using the pipette. Once the remaining 7 wells in that plate row were filled, each well received one drop of mineral oil to prevent oxidation of the sample. The row was then covered with masking tape to prevent contamination of the sample though aerosols and human error. The remaining rows were then loaded in the same way, making up a new master mix for each row of the plate prior to sample loading. Once completed the plate was analysed using a CEQ 8000 DNA analysis system (Beckman Coulter Inc, High Wycombe, UK).

The relative abundance of each peak occurring (within each sample) at a dye signal greater than 100 was included in assessment, with any shoulder peaks (associated with base pair addition through the use of PCR amplification) removed from analysis by grouping fragments with a band width of 1.25 bp (Edel-Hermann *et al*., 2004; Hodgetts *et al*., 2007). Firstly the number of peaks within each sample was determined for

assessment of the species richness within the sample. In addition, Simpson's diversity index was calculated using Equation 2.7 to allow assessment of the diversity within each sample for both bacterial and fungal datasets. Finally multivariate analysis using principal component analysis (PCA) was used to assess any trends within the datasets.

$$D = 1 - \left[\frac{n(n-1)}{N(N-1)} \right] \quad \text{(Eq. 2.7)}$$

Equation 2.7: Simpson's Diversity Index.
Where: n = the total number of organisms of a particular species
N = the total number of organisms of all species

2.3.4 MYCORRHIZAL COLONISATION

Mycorrhizal colonisation of *P. lanceolata* roots was determined following staining in Chlorazol Black E (Brundrett, Piche and Peterson, 1984). Roots were washed in de-ionised water and cut into 4 cm sections, before being cleared in 5 % KOH at 90 ^{o}C for 20 minutes. Once cleared the roots were removed from the KOH solution and rinsed with de-ionised water, before being stained for one hour at 90 ^{o}C in a 0.1 % Chlorazol Black E lactoglycerol solution containing equal volumes of 80 % lactic acid, glycerol and de-ionised water. After staining, the roots were transferred into glycerol for storage and destaining. Colonisation was quantified using the method of McGonigle *et al. (*1990) at 50X magnification to give percentage root length colonised (Figure 2.10).

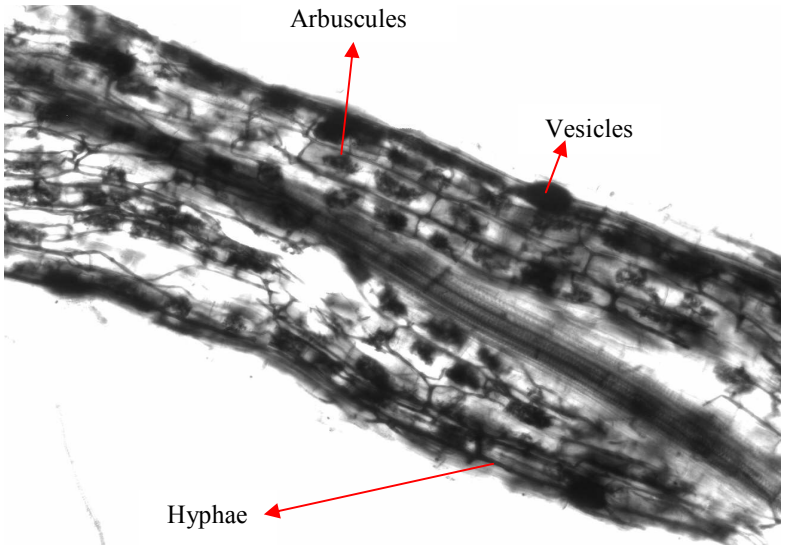


Figure 2.10: Colonisation of a *P. lanceolata* root with arbuscular mycorrhizal fungi observed after staining roots with chlorazol black E (50X magnification).

2.3.4.1 Mycorrhizal dependency

Mycorrhizal dependency (MD) was defined by Gerdemann (1975) as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility. The calculation described by Plenchette, Fortin and Furlan (1983) to determine the dependency of shoot growth on AMF (Equation 2.8) was used here as a measure of MD. An MD value > 0 suggests that a plant benefits from AMF, while an MD value < 0 means that the mycorrhizal fungi reduces plant growth under the prevailing environmental conditions (van der Heijden, 2002).

$$\text{Dependency of growth (MD)} = \frac{\text{Dry mass (+AMF)} - \text{Dry mass (-AMF)}}{\text{Dry mass (+AMF)}} \quad \text{(Eq. 2.8)}$$

Equation 2.8: Mycorrhizal dependency (Plenchette *et al*., 1983).
Where: +AMF = the presence of mycorrhizal fungi and –AMF = the absence of mycorrhizal fungi.

2.4 SHOOT AND ROOT BIOMASS

Shoot biomass was determined by cutting the plant material off at the soil level and rinsing in de-ionised water to remove any adhering soil particles. The plant material was then placed in an oven at 80 ^{o}C for 2-3 days to dry (or until a constant weight was reached). Root biomass was determined in a similar manner, with a subsection either stored in 70 % ethanol or at -80 ^{o}C for assessment of AMF colonisation (section 2.2.4).

3 EXPERIMENT 1: THE EFFECT OF MICROORGANISMS ON SOIL STRUCTURAL DEVELOPMENT.

3.1 INTRODUCTION

Soil structure develops as a result of numerous factors including wet-dry cycles, clay flocculation, root activity, burrowing by soil organisms, fungal hyphae and microbial exudation (as described in more detail in section 1.1.1). A defining feature of all soils is the spatial and temporal heterogeneity of water, substrates and microbial populations that can range across all scales from nm to km (Young and Crawford, 1998). Isolated pools of organic matter can exist within the soil environment, that can be accessed through tortuous pore pathways by microbial populations, acting as substrates for metabolic processes. Furthermore, the activity of microbial populations relies on the presence of water filled pores and how connected these soil pores are, particularly for microbial movement of bacteria, nematodes and protozoa. For gases, the requirement for replacement at the sites of this microbial activity requires pore connections to the air (Young and Crawford, 2004). In addition to the pore connectivity within a soil ecosystem, pore throat size can also influence the presence of microbes within a soil environment. Pores < 20 μm constitute a barrier to nematode movement (Wallace, 1958), whereas pores < 3 μm can inhibit protozoa (Kuikman, van Vuuren and van Veen, 1989).

Previous studies have found the dynamics of soil structure development to be closely related to the cycling of organic matter (Chaney and Swift, 1984; Oades, 1984; Jastrow, 1996; Six *et al.,* 2000; Six *et al*., 2004). As the soil surface becomes enriched

with organic matter, microbial activity increases in response, causing an increase in the size and stability of soil aggregates. As discussed by Tisdall and Oades (1982), bacteria, fungi and roots are very important binding and stabilising agents within the soil environment. They suggested that organic binding agents can be classified into three groups; transient, temporary and persistent. Transient binding agents (i.e. organic materials; in particular polysaccharides derived from microbes and plants) are readily decomposed, with their effects only lasting for a few weeks. In comparison, temporary binding agents, from roots and fungal hyphae can last for months and even years. Finally, degraded humic materials, associated with amorphous iron, aluminium and aluminosilicates are persistent binding agents.

Despite this understanding of how biotic factors can initiate aggregation over periods of weeks to years, there is very little literature regarding the specific timescales in which soil structure develops and/or aggregates form (including from completely disturbed soil environments). The problem of determining the time it takes for soil structure to develop depends greatly on the scale at which the soil is assessed. Changes in soil structure can be monitored at various scales, the micro-, meso- and macro-level. In light of this, it would be expected that development of soil structure at the micro-level (in terms of formation of aggregates and pores) would be more noticeable and faster than large structural changes at the field scale.

Feeney *et al.* (2006a) found the number of aggregates > 2000 μm and pore distributions within individual aggregates significantly increased (in addition to the total porosity) over a 30 day period, particularly in rhizospheric soil suggesting soil structure is

influenced by biotic activity extremely quickly. Water repellency also increased rapidly with increased active fungal populations, indicating that fungi released hydrophobic substances aiding formation and stabilisation of aggregates. In addition, porosity was shown to increase with the presence of roots and active bacteria, this yet again illustrates that root and microbial populations within the soil environment can rapidly alter porosity, pore geometry, water repellency and hence stability through their action.

De Gryze *et al. (*2005) found that aggregate formation within sandy loam, silt loam and silty clay loam soils, whose soil structure had been destroyed (by devastating structures > 53 μm in size) linearly increased with increasing amount of wheat residue after a three week period. These results provided initial data for a model proposed by these authors that suggested macro-aggregate turnover time was in the region of 40-60 days. This was somewhat longer than that found by Plante, Feng and McGill (2002) whose incorporation of ceramic microsphere to soil suggested macro-aggregate turnover of 4-33 days. More recently De Gryze, Six and Merckx (2006) replicated these timescales of macro-aggregate turnover showing it to be 30 days within a silt loam soil. Furthermore this research found macro-aggregate formation and re-stabilisation rates were faster after addition of microbial substrate, but then rapidly decreased, suggesting that changes in macro-aggregate formation rates followed changes in microbial activity. Turnover rates of micro-aggregates were 88 days showing that micro-aggregate formation occurred more slowly than that of macro-aggregates.

Langmaack *et al. (*2002) demonstrated that rehabilitation of degraded soil structure could take place within two years after a single compaction event. This was particularly

aided by the presence of earthworms, thus demonstrating the dynamic nature of the system and the need to understand the individual roles of soil fauna, microorganisms, roots, inorganic and physical processes on soil structure development.

Although the effects of various factors including organic matter, clay mineralogy, mycorrhizal fungi, roots and microbial communities on soil aggregate formation have been previously investigated (Tisdall and Oades, 1982; Dexter, 1988; Chenu, 1989; Rillig, Wright and Eviner 2002; Marschner and Baunmann, 2003), the experiment described here represents the first evaluation of the development of soil structure over time within a bare soil environment (without the addition of organic matter). Therefore the aim of this experiment was to determine the time taken for soil structure to develop within microcosms of soil containing three different soil types. Soil structural development within this context was classed as increases in total porosity, aggregation and mean pore area within the microcosms over the incubation period. Such development would improve water, air and nutrient movement within the soil and hence microbial functions. The aim of this experiment is to test hyptheses one and two (section 1.5).

3.2 MATERIALS AND METHODS

3.2.1 MACROCOSM CONSTRUCTION

Unsterilised air dried soil (sieved < 2 mm) from each of the three different soil textural field sites as described in section 2.1.1 (Newport, Worcester and Dunnington Heath),

were packed loosely into plastic cores (7.6 cm length x 2.44 cm width). These cores had 400 μm mesh (Cadisch Precision Meshes Ltd, London, UK) glued to the bases to allow capillary rise of water from capillary matting (Figure 3.1).

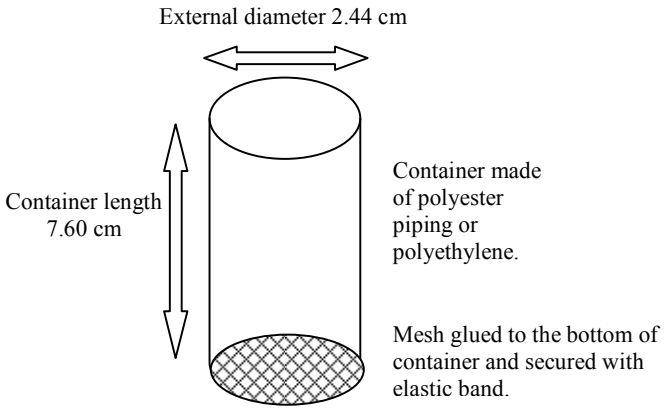


Figure 3.1: Soil microcosm used within the experiment.

3.2.2 SOIL INOCULATION AND REPLICATION

Soil microcosms were inoculated using a soil slurry solution, made from field fresh soil (taken from each respective field site (section 2.1.1) on the day it was required) using a soil slurry solution made to a dilution of 10^1 (Salonius, 1981; Griffiths *et al.,* 2001). Soil inoculum originating from each of the respective field soils was re-introduced into the microcosms to compensate for any reduction caused by the air drying and sieving procedures conducted prior to packing the microcosms. At inoculation the soil microcosm were left to saturate in the 10^1 soil slurry solutions of each respective soil type overnight as described in section 2.1.2. There was a total of 27 microcosms, one per soil type for each sampling date including an additional set for X-ray μCT and three replicates (for each soil type) for biological thin section analysis at the end of the investigation. The key aim for this investigation was to study the soil structure changes

overtime. Logistics prevented image analysis of more than one microcosm per soil type per harvest, using the novel imaging approach here. Microcosms were maintained in a glass-fronted incubator at 16 ^{o}C throughout the investigation without any additional lighting supplied. Soil moisture was determined by regular weighing and microcosms were maintained at field capacity through the addition of deionised water.

3.2.3 HARVEST REGIME AND MEASUREMENTS

A subset of the soil microcosms was destructively harvested at time zero, three, six, nine and fifteen months after inoculation for structural assessment. Microbial populations were also quantified at each harvest date, except for time zero. At each harvest, soil was gently removed from the microcosms to investigate cultivable microbe populations by culturable plate counts and Biolog microtitre plates (section 2.3.1). Biolog plates were only analysed after 96 hours from inoculation. At each harvest three pseudo-replicates of soil were sampled from each microcosm, which allowed assessment of the variability within each microcosm. For non-destructive soil structural assessment, one column was scanned by X-ray μCT at each harvest period (for each soil type) although results from the third month are not shown due to image resolution issues. Further biophysical measurements were made at the final fifteenth month harvest though the use of biological thin sections. Most methods undertaken in this experiment were described earlier in chapter 2; with a full list of the measurements undertaken in this experiment give in Table 1.3. Additional measurements solely used in this experiment alone have been described below.

3.2.4 *CULTURABLE MICROBIAL COUNTS*

Culturable soil bacteria and fungi were determined by removing the soil from each microcosm into a plastic bag and homogenising. The soil was then stored at 4 ^{o}C for no longer than 24 hours. Soil moisture content was determined by taking 3-7 g of soil from each core into a crucible and oven dying at 105 ^{o}C until at constant weight. At each harvest period 2 g dry weight equivalent of fresh soil was weighed aseptically into 250 ml sterile duran bottles. To this soil 198 ml of sterile ¼ strength Ringers solution (section 2.1.2) was added to each bottle to create a 10^2 soil suspension. This suspension was shaken and left to settle for 1 minute before being vortexed for 20 seconds to dislodge bacterial cells from the soil particles. The supernatant was serially diluted (standard 10-fold dilutions) with sterile ¼ strength Ringers solution and 0.2 mL aliquots of each dilution placed onto Petri dishes containing tryptic soy agar (TSA) (Oxoid, Basingstoke, UK) or potato dextrose agar (PDA) (Oxoid) amended with 100 μg ml^{-1} (w:v) streptomycin (Sigma, Poole, UK). Streptomycin solution was added to the agar to prevent bacterial growth (Pepper, Gerba and Brendecke 1995). All agar was made up according to the manufacturer's instructions. Plates were incubated at room temperature (~ 20 ^{o}C) and monitored daily for colony growth before enumeration at five days after inoculation. TSA was selected following recommendations by Vieira and Nahas (2005).

3.2.5 *BIOLOGICAL SOIL IMAGING*

At the final harvest, fifteen months after inoculation, three microcosms from each soil type were stained using SCRI Renaissance 2200 which highlights active

microorganisms (particularly fungi). The samples were fixed with 2 % glutaraldehyde (to stop microbial activity) and stained using 0.2 % aqueous solution of SCRI Renaissance 2200 (Renaissance Chemicals Ltd, Selby, UK), for 1.5 hours under vacuum as described by Harris *et al. (*2002). Once fixed and stained, the samples were impregnated, as one large block, with an epoxy resin mix. Resin solutions were made using a 1:1 v:v mix of thinned 'crystic' epoxy resin (Crystic 17449, Aeropia Ltd, UK) and acetone (Laboratory Reagent Grade, Fisher Scientific, UK). Catalyst (Organic peroxide '0' – Methyl Ethyl Ketone Peroxide, ScottBader, UK) was used in a 100:1 v:v ratio of resin to catalyst with cystic accelerator 'G' (Aeropia Ltd, UK) used in a 100:0.2 v:v ratio of resin to accelerator. This mixture was slowly poured over the soil samples (that were housed in a large plastic container), until it completely covered each sample by 1 cm. The samples (within the plastic container) were then placed under a low vacuum to evacuate air within the soil microcosm. Extra resin mixture was used to top up the samples as the resin level dropped below the soil microcosm surface. Soil blocks were cured until solid at room temperature, before curing for an additional two weeks at 40 ^{o}C.

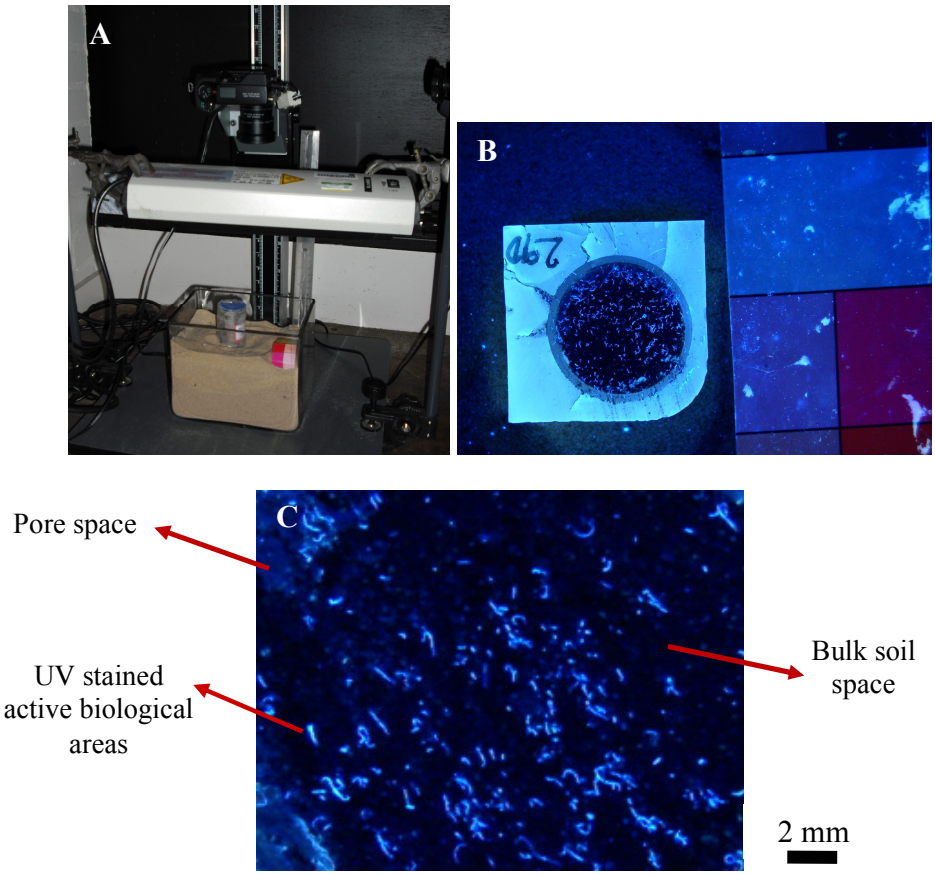


Figure 3.2: Images showing the UV imaging of resin impregnated soil segments. A) The imaging set-up using UV lamp, camera and sand tank containing sample; B) a fluorescent soil segment surface after cutting with a diamond saw; C) example of cropped image taken from a soil segment showing microbial activity, bulk soil and some pore space.

The cured soil block (containing each microcosm from each soil type) was cut using a diamond saw, to remove individual microcosms at specific column depths (12, 28, 44 and 60 mm from the top of the soil surface). This exposed area of the soil block, when viewed under a UV light, is highly fluorescent due to the microbes present within the soil. Cut soil faces were wiped clean using acetone to remove dust from block cutting. Images of each soil segment were taken under darkroom conditions prior to processing. Photographs of soil samples were taken using an Olympus Camedia C-4000 Z digital camera and an ultra violet light source (UVP – Model UVL-28 assembly, long wave,

230v, 50 Hz, 0.32 Amps) set at a constant distance from the soil sample surface to maintain constant resolution (Figure 3.2). The camera was set to macro lens; full zoom (3X optical); no flash; image size 1600 x 1200 pixel; and TIFF (tagged image format). A Raynox RT5241 F52-M41 mm UV filter was attached to the camera lens to prevent over exposure under the UV light and a Kodak colour chart was placed in the field of view for image analysis calibration (Figure 3.2). Images were transferred to computer for processing using digital media cards.

3.2.6 IMAGE PROCESSING AND ANALYSIS OF BIOLOGICAL SOIL SEGMENTS

Image manipulation was undertaken using analySIS® (Soft Imaging Systems (SIS), Munster, Germany) to isolate areas within the soil microcosm showing signs of namely fungal biological activity. The image resolution was 66.34 μm $pixel^{-1}$ and images were cropped to 15.31 x 15.09 mm in size. No colour filtering was applied to the images to prevent distortion of UV hotspots within the soil microcosms. Images were binarised with an auto colour threshold function (to remove bias) by spliting an RGB image into three 8-bit greyscale images containing the red, green and blue components of the original. The auto colour threshold was set in the following red, green, blue (RGB) colour ranges (Red 20 - 255; Green 0 - 255 and Blue 0 - 255) to highlight areas of microbial activity instead of pores (Figure 3.3).

Morphological analysis was performed on binary images using analySIS® to assess i) the total area of microbial activity in relation to the bulk soil; ii) the mean individual areas of microbial activity; iii) the equivalent circular diameter (ECD) showing the diameter of a circle that has an area equal to the area of microbial activity analysed; iv)

circularity/sphericity of the isolated area; v) the nearest neighbour distance to measure the average distance between the centres of microbial activity and finally vi) the mean perimeter of areas of microbial activity that is defined as the total pixel distance along the boundary of microbial activity.

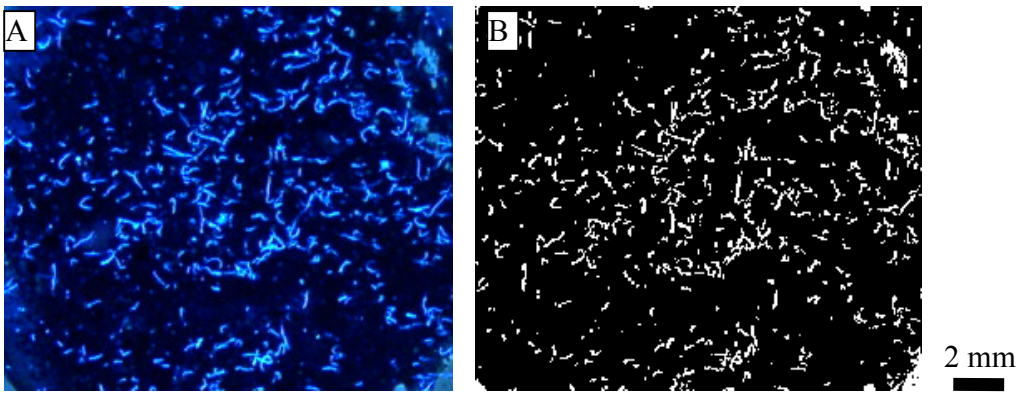


Figure 3.3: Example image of A) biologically stained soil segments and B) the binarised image of this soil segment showing biologically stained areas outside and inside pore spaces.
Note: Biologically active areas are light blue in figure A and white in figure B.

3.2.7 X-RAY μCT ASSESSMENT

Soil structure was determined in the cores by scanning undisturbed cores (for each soil texture) in a μCT scanner (μCT 40 Scanco Medical Scanner, Department of Engineering, University of Nottingham) with the following settings: 36 mm sample diameter high resolution scan, 70 kV, 112 μÅ, 39 mAs, and 150 ms. Microcosms were scanned in an area 20 mm from the top and base of the microcosm to reduce the effect of watering and surface disruption (Figure 3.4). Approximately 350 image slices were collected for each sample.

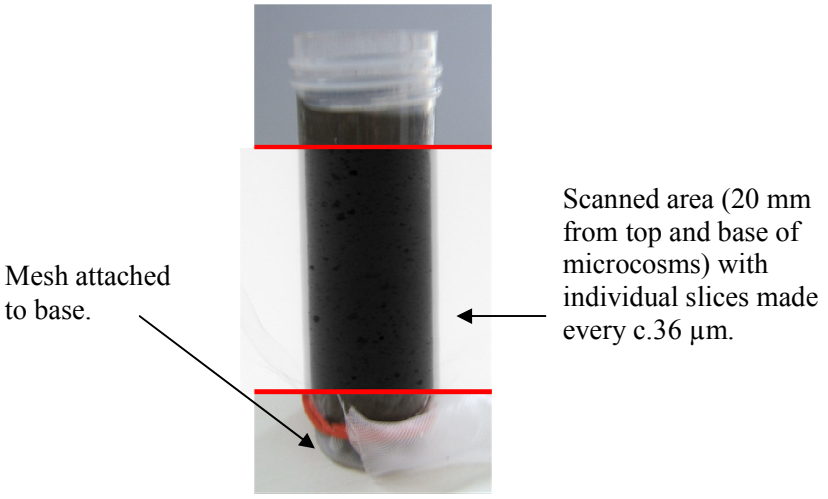


Figure 3.4: Soil microcosms with area selected for X-ray CT assessment.

3.2.8 IMAGE ANALYSIS

Images obtained using the CT scanner were processed and assessed using the public domain software ImageJ® (v 1.39u, National Institutes of Health, USA, http://rsb.info.nih.gov/ij/) to isolate pore spaces within complete microcosms (Figure 3.5). The final image resolution was set at 55 µm pixels $^{-1}$ at the time zero, sixth, nine and fifteen month scans. Throughout the experiment, it became apparent that cracking, particularly within the clay loam macrocosms, would influence porosity, mean pore size in addition to shape analysis of the pores within each macrocosm. It was therefore deemed appropriate to analyse a subsection of the image by reducing the analysed area within each stack to remove the influence of this cracking. As a result the image was cropped to 8.5 x 8.5 mm to prevent the cracking influencing the morphological measurements.

Filtering was performed on the image stack by using an enhance contrast function, that provided an adjustment of the contrast by enhancing the image using histogram equalization to the whole set of images within that scan; a second despeckle filter acted as a median filter, replacing each pixel with the median value in its 3 x 3 neighbourhood to reduce salt and pepper noise within the image; with a final smooth filter to remove noise further by replacing each pixel with the average of its 3 x 3 neighbourhood. Due to the inconsistency and over estimation by all automatic threshold algorithms available in ImageJ (i.e. Automatic, IsoData, Ostu and Maximum Entropy; Figure 3.6), thresholding was performed manually for each sample stack. From the manual threshold, automatic thresholds appeared to overestimate porosity between ~ 20-75 %. Binary images (stacks) were then subjected to binary erosion consisting of one iteration and five pixels to prevent over estimation of pore space present within the microcosms.

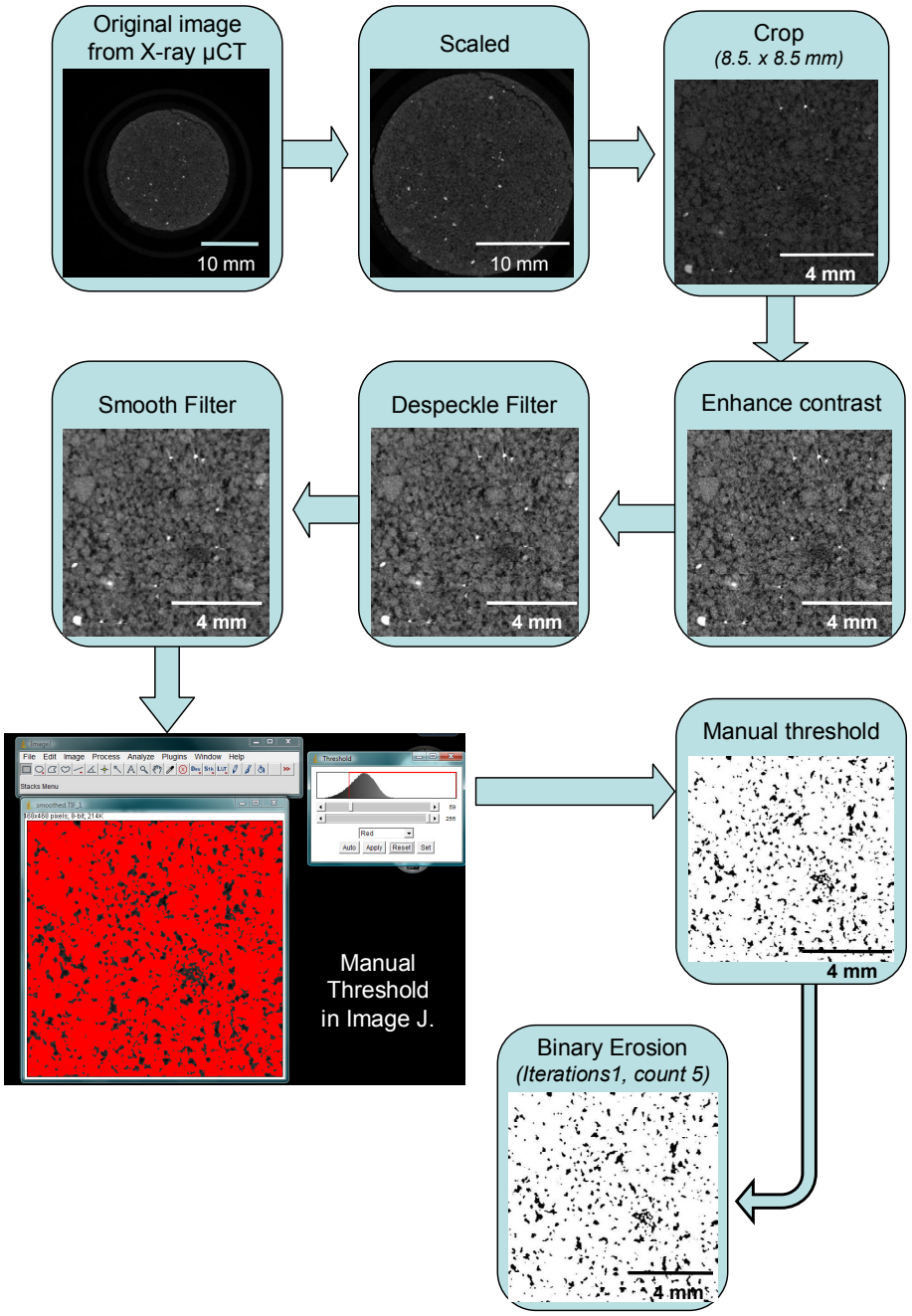
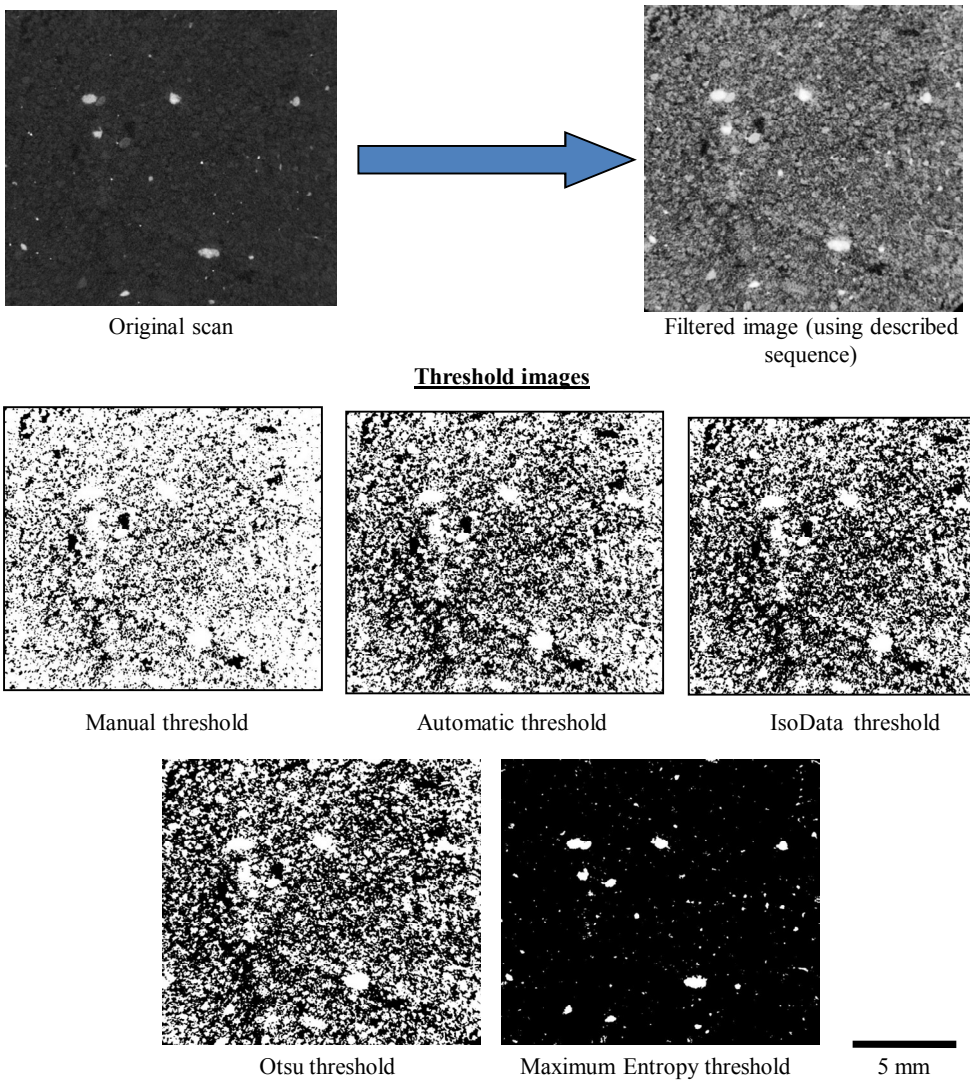


Figure 3.5: Details of each image stack manipulation stage using ImageJ.



Comparison of porosity of image slice (%)				
Manual	Automatic	IsoData	Otsu threshold	Maximum Entropy
22.93	42.51	48.07	55.00	97.80

Figure 3.6: Examples of Image J Manual, Automatic, Isodata, Ostu and Maximum entropy thresholds of individual images from the image stack; showing variations of total porosity of each image respectively.

Individual scans were checked for errors during scanning and reconstruction. It was apparent that radial scatter and machine errors were apparent in some slices at this stage. To prevent such scatter and error influencing results, these slices were withdrawn from analysis (Figure 3.7) to leave a total of 270 slices per image stack for each sample. Morphological analysis was performed using ImageJ; this allowed measurement on pore counts, total pore area, average pore size, total image porosity, pore size distribution, pore perimeter and circularity. Over the course of the investigation pore perimeter followed a close and significant relationship with mean pore area (Kampichler and Hauser, 1993; Pachepsky *et al.,* 1996), with larger pores having a larger pore perimeter. Due to the similar nature of this measurement, perimeter data have not been presented here.

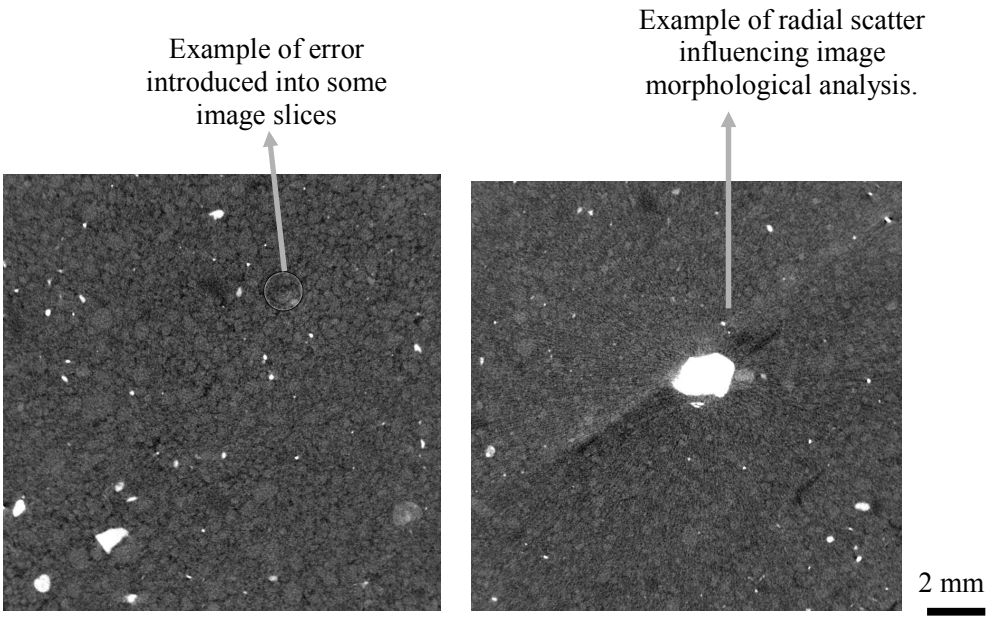


Figure 3.7: Example of artefacts introduced into individual stack slices.

3.2.9 STATISTICS

Background microbial numbers and activity were analysed within each microcosm. Analysis of variance (ANOVA) (Genstat 11.1) were performed on culturable counts (with CFU data transformed using a $\log_{10}$ transformation) and Biolog data (percentage substrate utilisation) using pseudo-replicates (3 measures per microcosm). With data taken from each harvest period, analysis was undertaken using a repeated measure ANOVA. Clearly these analyses should be viewed with caution, but are nevertheless useful in giving an indication of the variability within each microcosm. This is important in helping determine any changes in soil structure overtime. Metabolic potential (Biolog data) was predominantly analysed by descriptive means (principal component analysis (PCA), using Genstat 11.1. Covariate PCA for each soil type and harvest combined were carried out on the background corrected average well colour development values that had undergone a natural log transformation using Genstat 11.1. Principal component (PC) scores were analysed using repeated measures ANOVA in Genstat. These analyses are therefore more robust than those of the culturable counts.

Repeated measures ANOVA was conducted on all pore measurements determined from X-ray μCT analysis at each harvest period using soil type as a factor, since the same macrocosms were scanned (at the same depth) over the investigation.

3.3 RESULTS

3.3.1 CULTURABLE MICROBIAL COUNTS

Greater variability in bacterial numbers over the fifteen month investigation was observed within the clay loam and loamy sand than within the sandy loam. Bacterial numbers in the sandy loam were relatively constant throughout (soil type x harvest interaction, $F_{8,26} = 6.66$, $P < 0.001$; Figure 3.8). Within the clay loam soil, bacterial numbers where highest at one month and lowest at three and fifteen months, whereas within the loamy sand bacterial numbers declined at six months. In terms of single factors bacterial numbers were highest in the sandy loam with fewest culturable bacterial numbers in the loamy sand soil ($F_{2,26} = 4.57$, $P = 0.020$). Harvest period also influenced culturable bacterial numbers with numbers higher (but not significantly) at the first and ninth month harvests ($F_{4, 26} = 2.39$, $P = 0.077$).

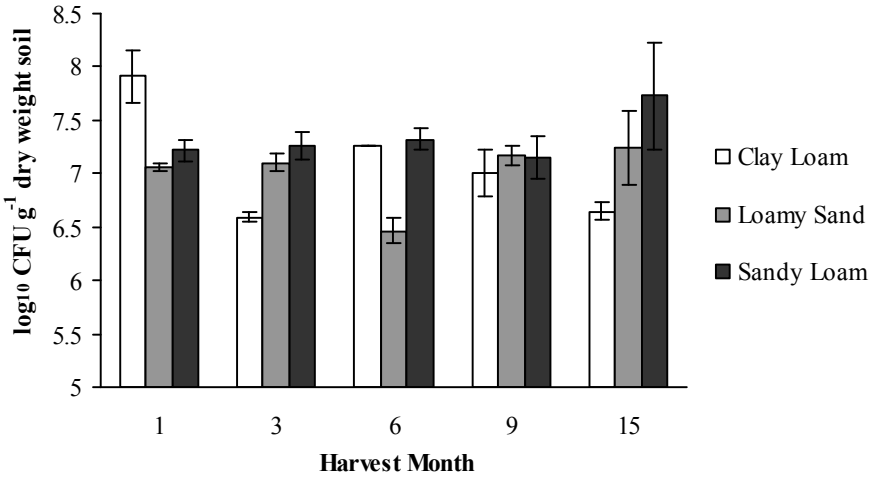


Figure 3.8: Effect of soil type and harvest period on numbers of culturable soil bacteria on TSA agar.
Standard error bars show variability within each individual microcosm (log_{10}).

More variability in fungal numbers over the fifteen month investigation was observed within the clay loam and sandy loam than within the loamy sand (soil type x harvest interaction, $F_{8,28} = 2.86$, $P = 0.018$; Figure 3.9). Within the clay loam and sandy loam, fungal numbers were highest at one and nine months compared to the other harvest periods. In terms of single factors, generally fungal numbers were significantly higher in the clay loam (5.9 [log_{10}] CFUs) and sandy loam (5.7 [log_{10}] CFUs) with least fungal numbers observed in the loamy sand (5.5 [log_{10}] CFUs) ($F_{2,28} = 30.80$, $P < 0.001$). Harvest period also influenced culturable fungal numbers with numbers higher at the first and ninth month harvests ($F_{4, 28} = 18.85$, $P < 0.001$).

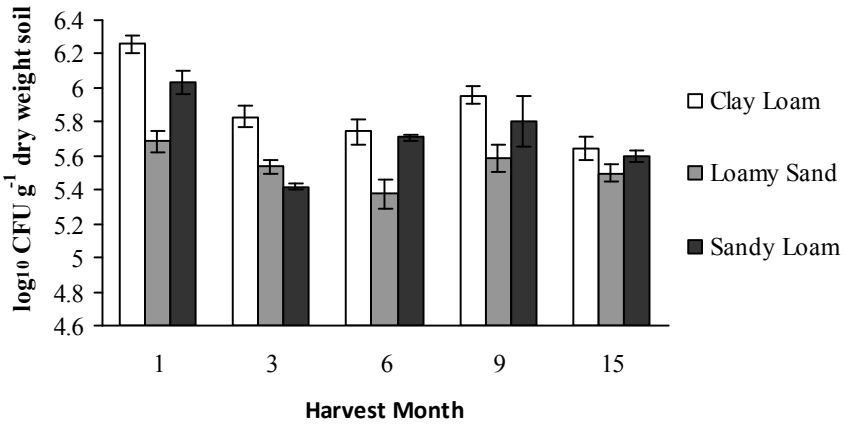


Figure 3.9: Effect of soil type and harvest period on total culturable soil fungi determined from PDA agar amended with cycloheximide.
Standard error bars show variability within each individual microcosm (log_{10}).

3.3.2 SOIL METABOLIC COMMUNITY ANALYSIS

Both soil type and harvest period had a significant effect on total substrate utilisation as determined through Biolog microtitre plates. Fewer substrates were utilised by bacteria

extracted from the clay loam soil than from the other soil types ($F_{2,28} = 4.59$, $P = 0.019$) particularly with increasing age of microcosms ($F_{4,28} = 33.39$, $P < 0.001$; Figure 3.10).

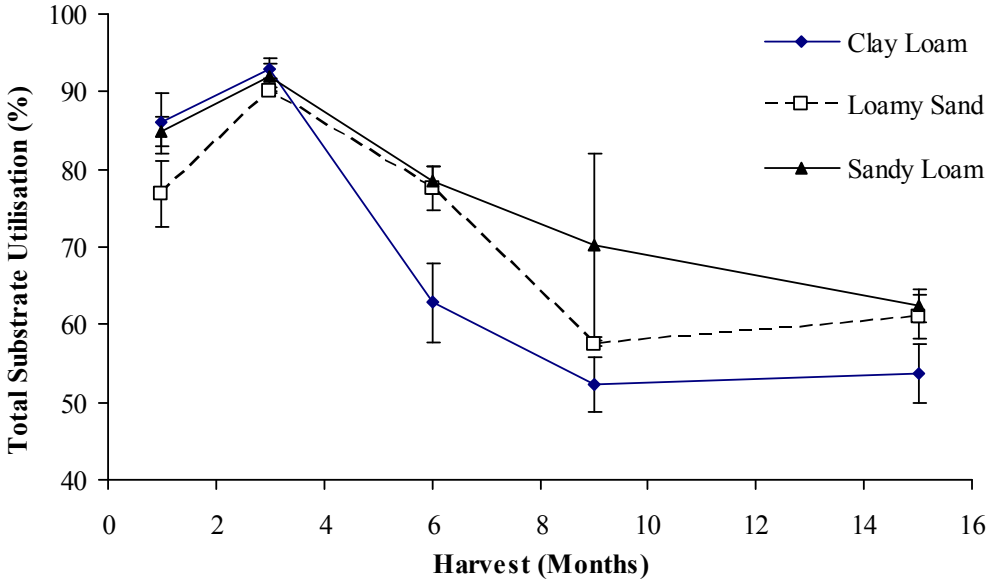


Figure 3.10: Total substrate utilisation for each soil type at each harvest period after 96 hours of incubation.
Standard error bars show variability within each individual microcosm.

Principal component analysis (PCA) of background-corrected AWCD (natural log +1) values for all 95 carbon sources on the Biolog GN2 microtitre plate was performed on data collected after 96 hours incubation for all sample dates combined. The first principal component (PC1) accounted for 84.14 % of the total variation, with the second component (PC2) accounting for 4.44 % of the variation (Table 3.1). PC1 and PC2 accounted for 88.58 % of the total variation; hence other PCs were sufficiently small to be ignored from analysis.

Table 3.1: Results of principal component analysis of background-corrected natural log transformed AWDC (+1) values for all 95 carbon sources in Biolog GN2 microtitre plates for each soil types at each harvest period.

NB: Latent root or eigenvalues are the sum of the squared factor loads for any given factor and can be thought of as the amount of variance for that factor.

	PC1	PC2
Latent root	1.6696	0.0882
% Variance	84.14	4.44
Cumulative	84.14	88.58

PC1 and PC2 loadings for each substrates are shown on separate axes (Figure 3.11). All PC1 loadings were negative; therefore the main axis of variation is one where the colour intensity of all substrates decreases or increases in unison. However, PC1 loadings do seem to vary in magnitude with substrate type; amino acids, carbohydrates, polymers and amines were more negative than bromidated chemicals, phosphorylated chemicals and alcohols. PC1 loadings significantly varied with substrate guilds, hence the variation between groups is greater than within each substrate group (ANOVA of PC1 loadings; $F_{10,84} = 2.16$, $P = 0.029$). However, PC2 loadings are both positive and negative. PC2 loadings did not vary significantly by substrate type ($F_{10,84} = 1.82$, $P = 0.069$), hence showing greater variation within substrate groups than between. Differentiation therefore, occurs between substrates with positive PC2 scores that are utilised and substrates with negative PC2 scores that are not utilised. In terms of guilds, amino acids, alcohols, amines and carboxylic acids are mainly positive, whereas amides, aromatic chemicals and phosphorylated chemicals are negative. Therefore amino acids, alcohols, amines and carboxylic acids were utilised, amides, aromatic chemicals and phosphorylated acids were not. Carbohydrates and polymers substrates are positive and negative, showing that certain individual C sources were preferred over others within substrate guilds.

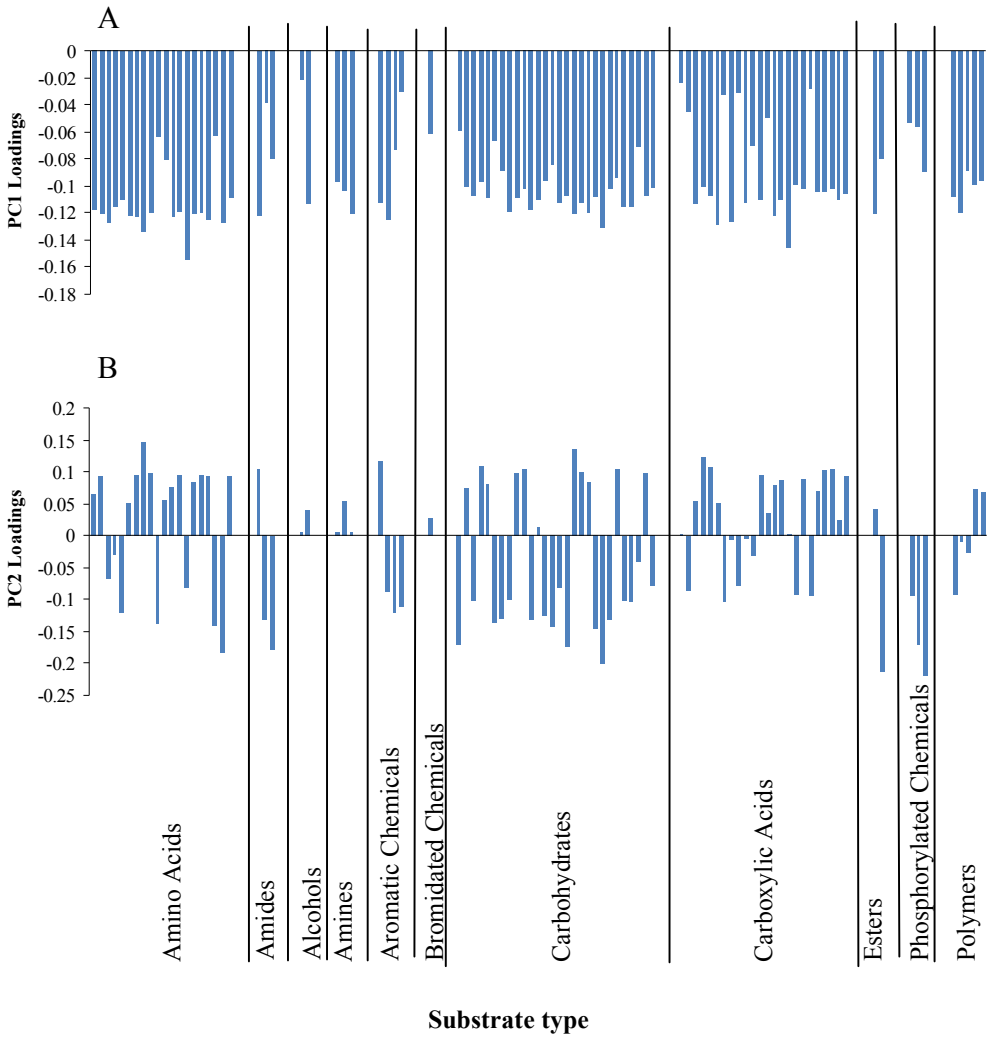


Figure 3.11: PC loadings for A) PC1 and B) PC2 of background-corrected AWCD (natural log transformed) data from the 95 carbon substrates in Biolog GN2 microtitre plates after 96 hrs incubation.

Principal component scores for the samples were analysed by ANOVA, with soil type and harvest period as factors. Biolog substrate utilisation patterns were soil type specific for both PC1 ($F_{2,28}$ = 9.19, P < 0.001) and PC2 ($F_{2,28}$ = 251.67, P < 0.001). Clay loam soil had mainly positive PC1 scores, whereas loamy sand had the most negative PC1 scores. PC1 scores for the sandy loam soil were significantly lower than for the clay loam soil (Figure 3.12). More negative PC1 scores account for greater colour development; therefore the bacterial communities originating from the clay

loam were producing less colour in the Biolog plates than communities in the sandy loam and loamy sand (corroborating Figure 3.10).

The loamy sand had positive PC2 scores, in comparison to the sandy loam and the clay loam soil which had predominantly negative PC2 scores (Figure 3.12). This suggests that within the loamy sand there was preferred utilisation of amino acids, alcohols, amines and carboxylic acids (i.e. substrates showing positive PC2 loadings); whereas the bacteria from the sandy loam and clay loam soils (which had negative scores), utilised mainly amides, aromatic chemicals and phosphorylated acids.

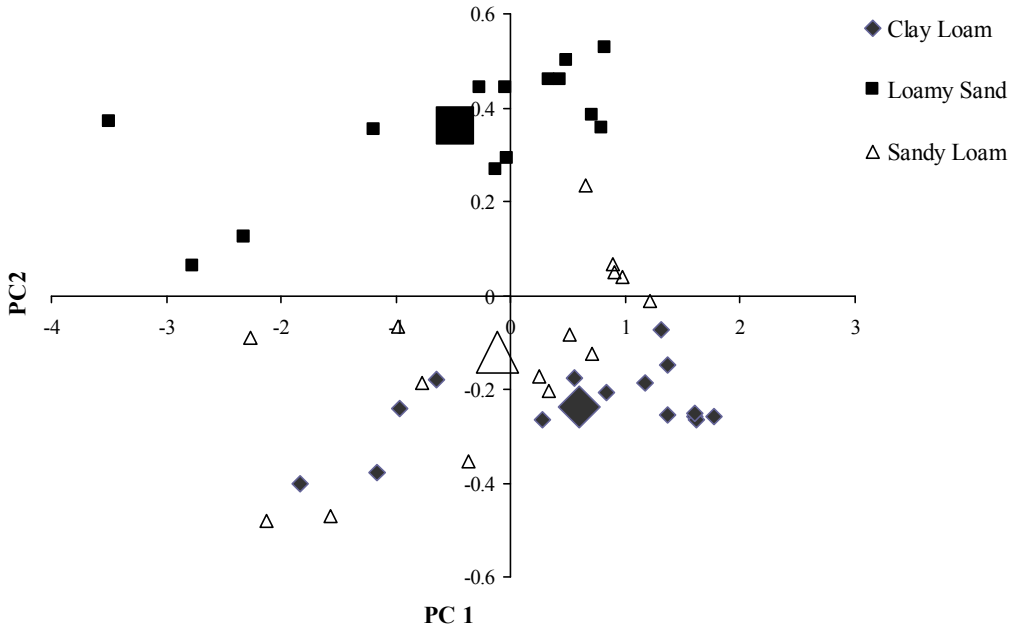


Figure 3.12: Ordination plot of PC scores produced from principal component analysis of transformed AWCD data of Biolog GN2 microtitre plates inoculated for 96 hrs for each soil type.
Large symbols represent mean PC centroids.

Biolog substrate utilisation patterns were also harvest period dependent for both PC1 ($F_{2,28}$ = 24.04, P < 0.001) and PC2 ($F_{2,28}$ = 20.26, P < 0.001) as seen in Figure 3.13. The third month harvest had the most negative PC1 scores. The first month harvest also had a negative PC1 score which was lower than the sixth, ninth and fifteenth month harvest which had positive PC1 scores (and which increased in value respectively). This once again followed the pattern that more negative PC1 scores accounted for greater colour development; therefore the highest substrate utilisation was observed in the third month (Figure 3.13). PC2 scores were highest at the fifteenth and ninth month with the only negative PC2 score found at the third harvest. At the ninth and fifteenth month harvest PC2 scores were mainly positive, suggesting there was preferred utilisation of amino acids, alcohols, amines and carboxylic acids. Since the first and sixth harvest PC2 scores were close to zero, this suggests there was no bias towards any particular C source utilised, whereas at the third month harvest utilisation of amides, aromatic chemicals and phosphorylated acids were preferred.

The effects of harvest period and soil type on PC1 and PC2 scores are shown in Figure 3.14. There was no harvest x soil type interaction for PC1 scores ($F_{8,28}$ = 1.28, P = 0.294). PC2 scores were highest in the loamy sand soil at all harvest periods (soil type x harvest interaction, $F_{8,28}$ = 4.25, P = 0.002) suggesting preferential utilisation of amino acids, alcohols, amines and carboxylic acids substrates. PC2 scores for the clay loam remained relatively constant over the experimental period but those of the sandy loam increased from 9 months onwards. This suggests a shift in utilisation of amides, aromatic chemicals and phosphorylated acids at to amino acids, alcohols, amines and carboxylic acids substrates in the later months.

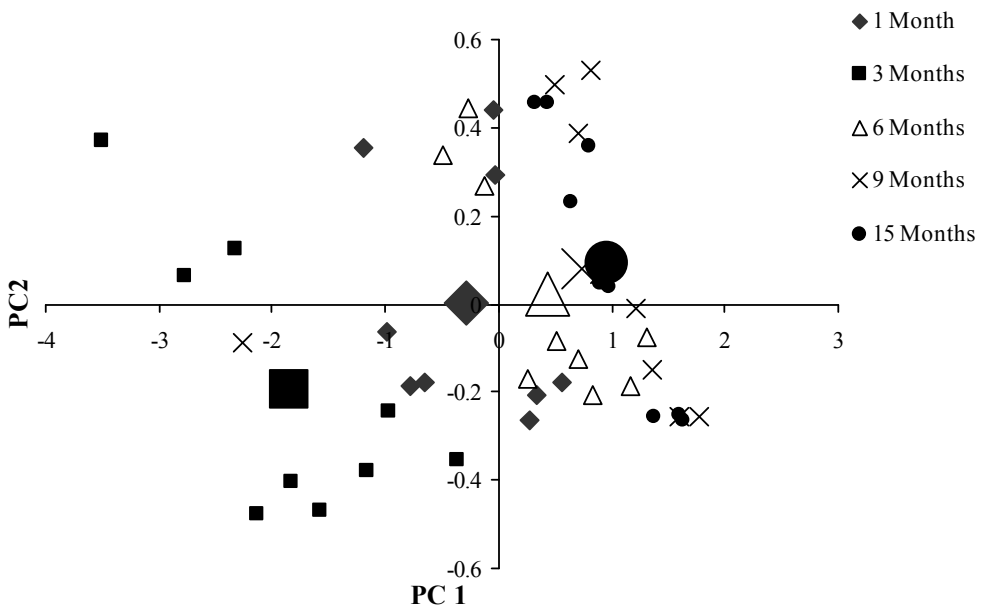


Figure 3.13: Ordination plot of PC scores produced from principal component analysis of transformed AWCD data of Biolog GN2 microtitre plates inoculated for 96 hrs for each harvest period.
Large symbols represent mean PC centroids.

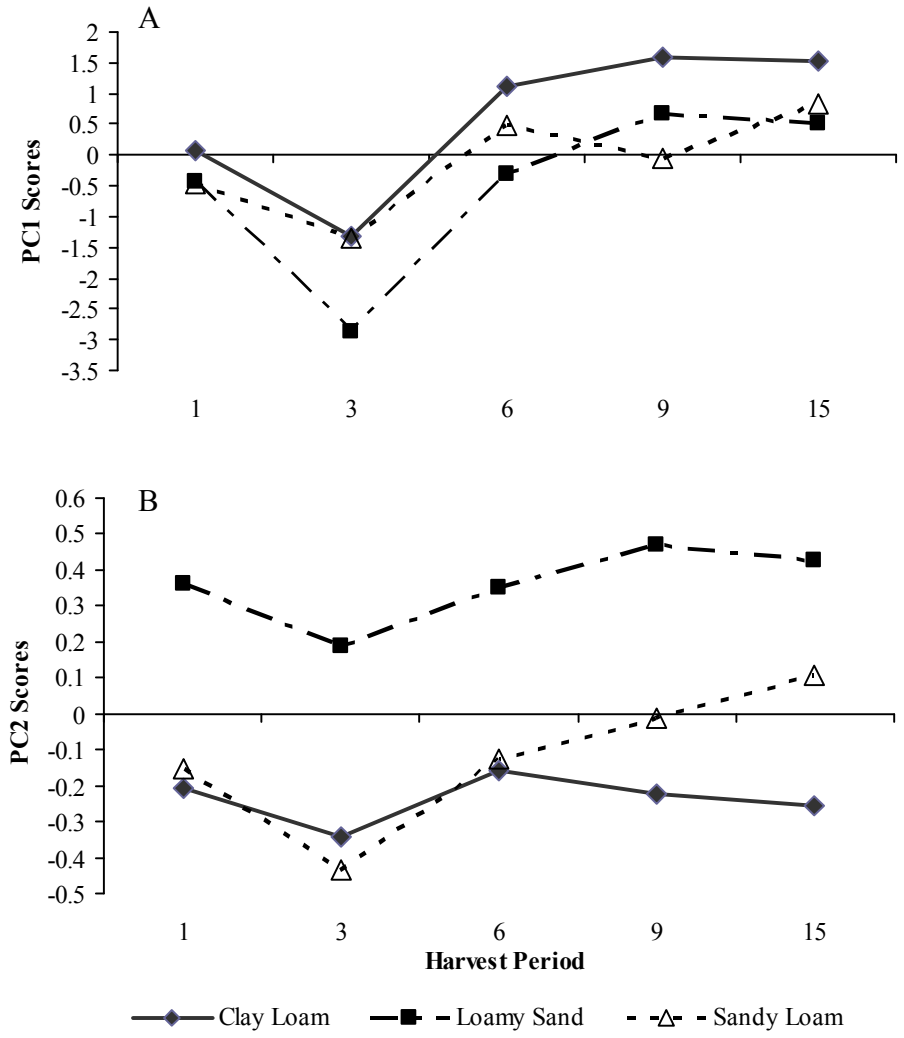


Figure 3.14: Interaction of soil type and harvest period for A) PC1 and B) PC2 scores, results.

3.3.3 MESOSCALE VISUAL EVALUATION OF SOIL STRUCTURE

The assessment of image stacks of size 8.5 x 8.5 mm allowed the removal of cracks, particularly within the clay loam microcosms from the morphological assessment of soil structure within the microcosms. This was most probably caused by wetting and drying taking place within the columns, causing shrinkage and swelling in the clay (Pires, Bacchi and Reichardt, 2005), despite the careful water regimes.

Generally pore spaces were fewer within the clay loam soil, than in both the loamy sand and sandy loam. Within the clay loam (Figure 3.15), the majority of pores present at the first harvest (time zero) were introduced due to the packing of the soil during microcosm construction. As time passed the pore spaces became smaller and more uniformly distributed within the soil at the third, sixth and ninth month harvests without any significant clumping of pores within a particular area.

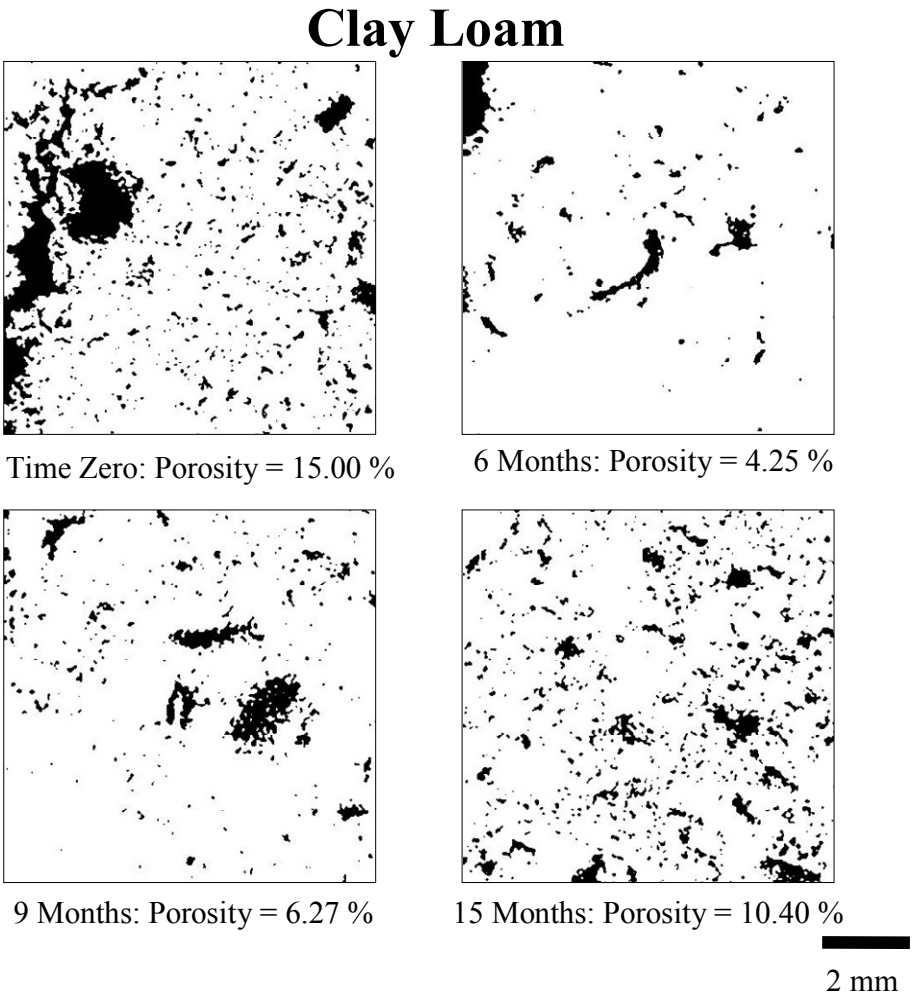


Figure 3.15: Example of pore space within clay loam microcosms over each harvest period (sample size = 8.5 x 8.5 mm). Porosity values given show total porosity of image, which is representative on the entire sample.

Throughout the investigation pore spaces within the loamy sand microcosms were similar over the course of the experiment (Figure 3.16), with an even distribution of pore space. At fifteen months porosity appeared to increase, with slightly larger pores present.

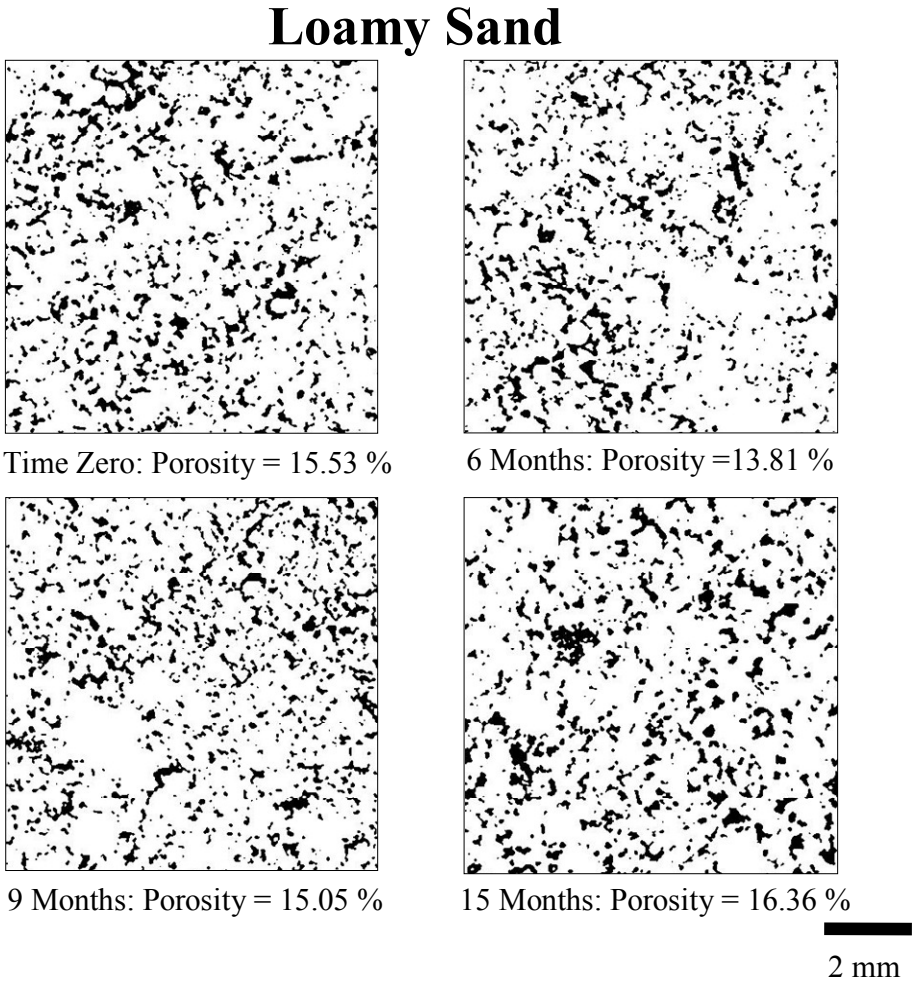


Figure 3.16: Example of pore space within loamy sand microcosms over each harvest period (sample area 8.5 x 8.5 mm). Porosity values given show total porosity of image, which is representative on the entire sample.

At the first (time zero) harvest slightly more pore space was present than at other harvest periods with the sandy loam, this possibly associated with packing conditions

(Figure 3.17). Over the investigation, the quantity of pore space within the sandy loam remained relatively constant with larger pores observed in the microcosm at the final fifteenth month in comparison to other stages of assessment.

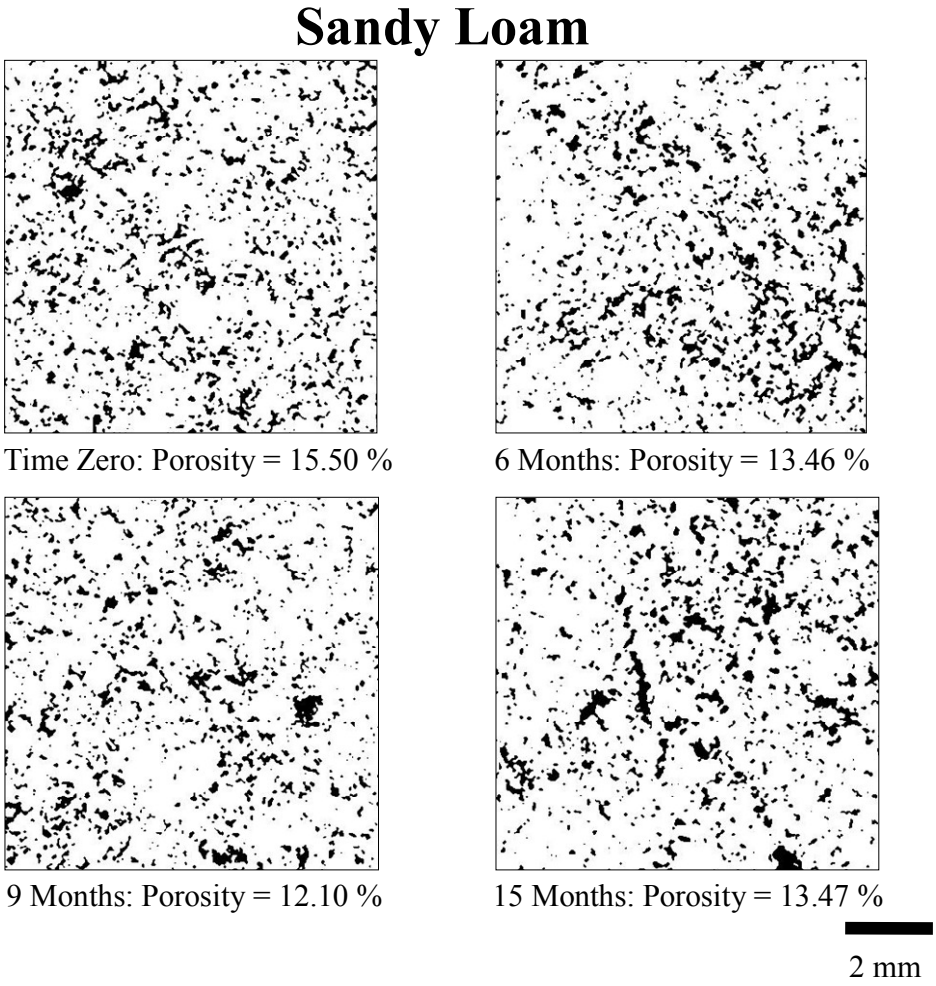


Figure 3.17: Example of pore space within sandy loam microcosms over each harvest period (sample area 8.5 x 8.5 mm). Porosity values given show total porosity of image, which is representative on the entire sample.

3.3.4 TOTAL POROSITY

Total porosity was significantly higher in the loamy sand (15.2 %), and lower in the clay loam (9.0 %) microcosms (soil type effect, $F_{2,2959} = 4709.57$, $P < 0.001$). Harvest

period also had a significant impact with porosity highest at time zero (15.3 %) than at fifteen months (13.4 %) and nine months (11.1 %) with total porosity found to be significantly lower at six months with a porosity of 10.5 % ($F_{3, 2959} = 1655.42$, $P < 0.001$).

Introduced porosity from packing (measured at time zero) was lowest in the clay loam microcosm (15.0 %) compared to the other two soil types, due to differences in particles size distribution associated with each soil type (soil type x harvest interaction, $F_{7,2959} = 624.68$, $P < 0.001$; Table 2.1; Figure 2.2; Figure 3.18). However, these differences were negligible. A reduction in porosity occurred after six months within the microcosms due to settling of the soil after loose packing. Changes in porosity overtime were most notable within the clay loam soil and loamy sand where there was a marked increase in porosity (144.7 % and 18.5 % respectively between the sixth and fifteenth month) compared to the sandy loam soil.

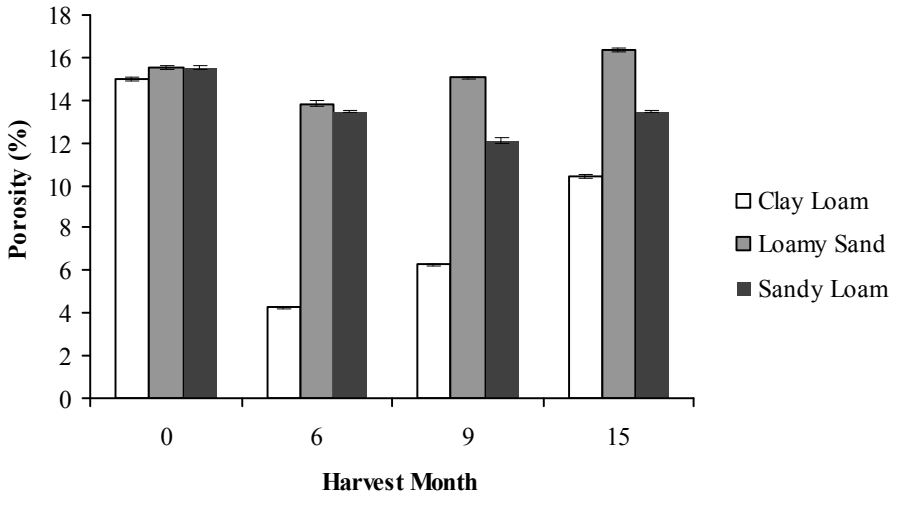


Figure 3.18: Total porosity of the 270 slice image stacks for all soil types at each harvest period ($F_{7,2959} = 624.68$, $P < 0.001$).
Standard error bars show variability within each individual microcosm.

3.3.5 *PORE SIZE DISTRIBUTION*

Within the clay loam soil at time zero (Figure 3.19) the pore size distribution (PSD) was normally distributed with pores 0.0316-0.1 mm^2 in size dominating. PSD at the sixth and ninth month harvest was uniform with a small and similar number of pores in all size groupings. At the final harvest period, the number of pores within the microcosm increased, with dominance of pores 0.01-0.1 mm^2 in size.

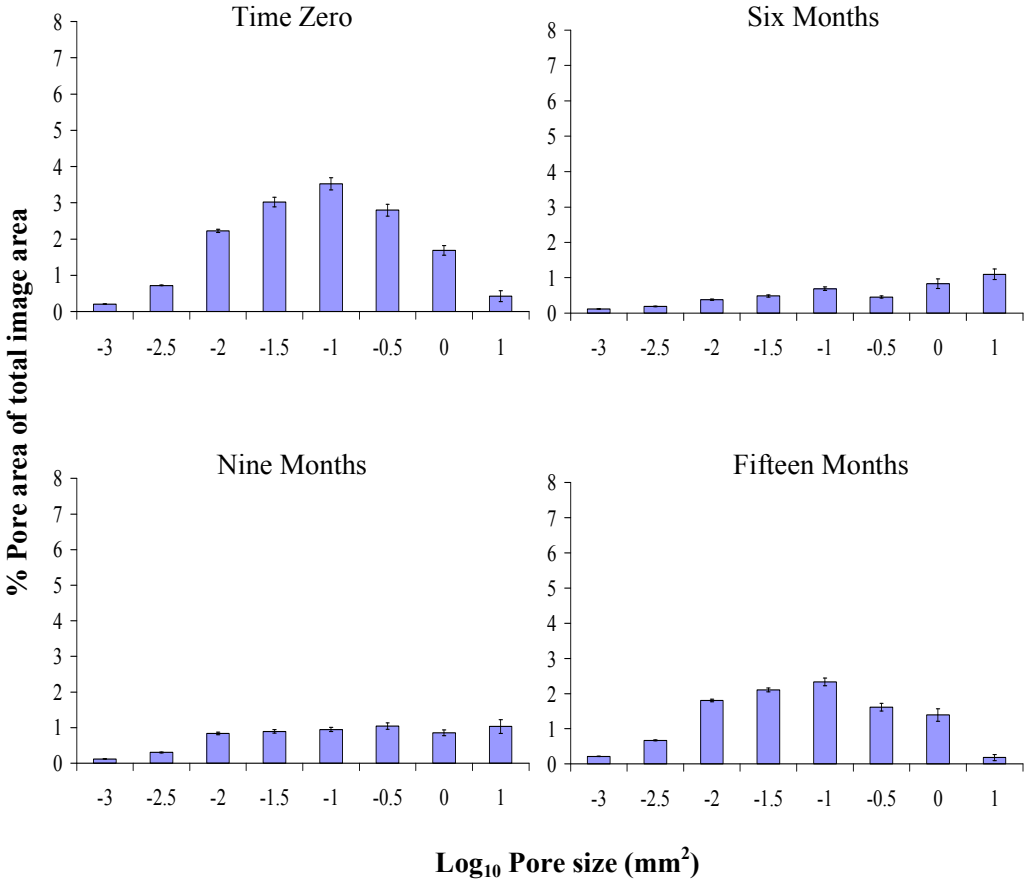


Figure 3.19: Mean pore size distribution for the clay loam microcosms over each harvest period, with image resolution of 55 µm $pixel^{-1}$.
Standard error bars show variability within each individual microcosm.

Within the initial time zero harvest of the loamy sand microcosms (Figure 3.20) pores of 0.0316-0.1 mm^2 in size dominated the system. A change in the PSD was observed at

the six month harvest with no pores > 10 mm^2 in size observed compared to the time zero scan. At the sixth and ninth month harvest the PSD is dominated by pores 0.0316-0.1 mm^2 in size. In comparison at fifteen months pores 0.1 mm^2 in size dominated the system.

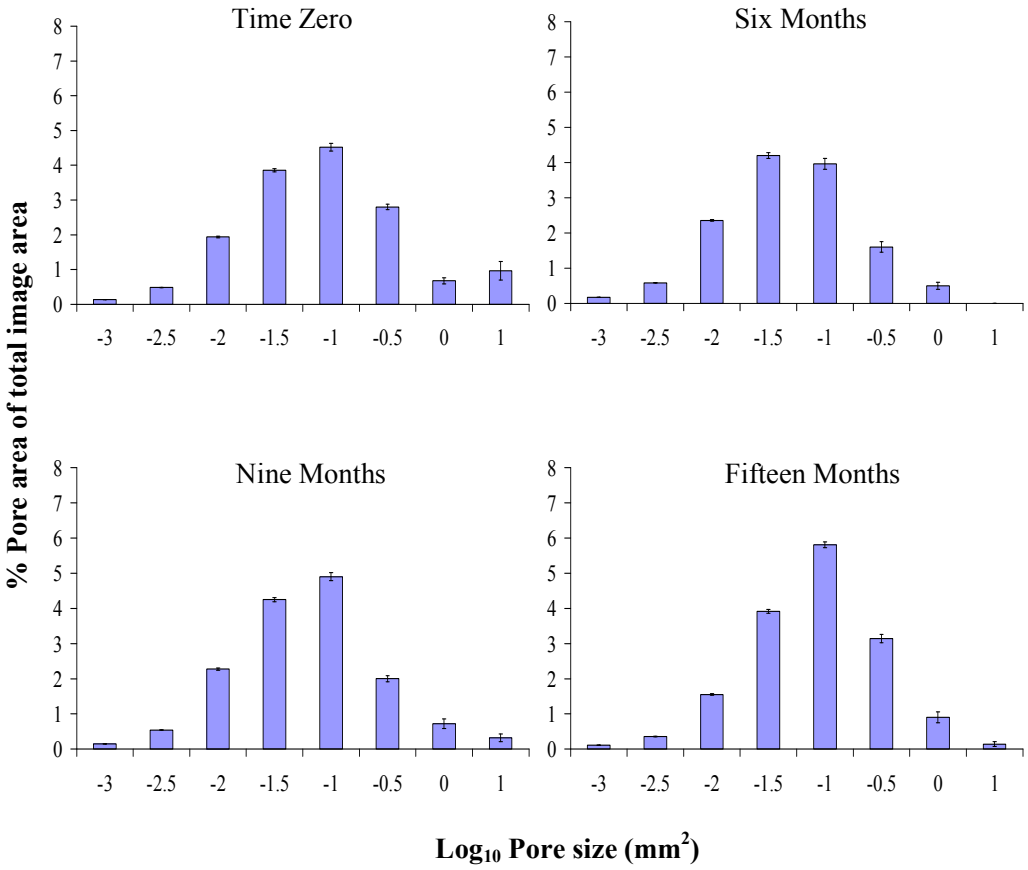


Figure 3.20: Mean pore size distribution for the loamy sand microcosms over each harvest period, with image resolution of 55 µm pixel^{-1}.
Standard error bars show variability within each individual microcosm.

The PSD for the sandy loam soil (Figure 3.21) was very similar over the initial time zero, six and nine month harvest, with pores 0.01-0.0316 mm^2 in size dominating the system. At three and fifteen months the PSD had shifted slightly, with the dominance of pores 0.0316-0.1 mm^2 in size dominating the microcosm. Although generally over the entire investigation the PSD hardly changed.

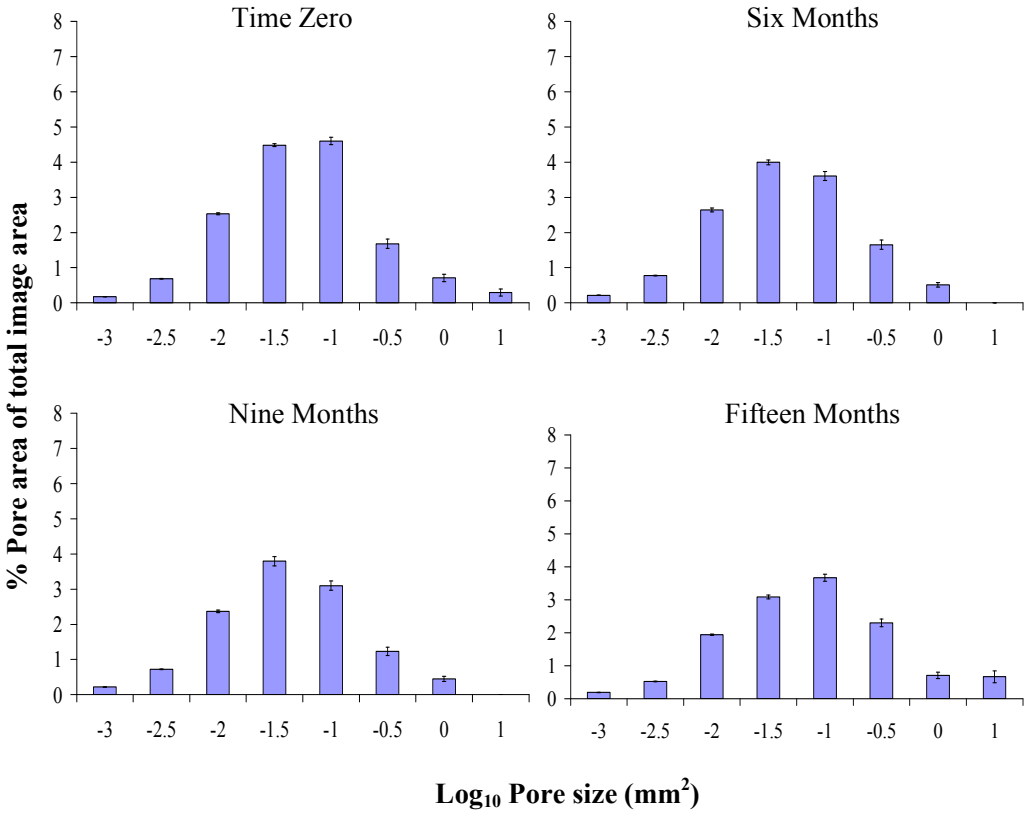


Figure 3.21: Mean pore size distribution for the sandy loam microcosms over each harvest period, with image resolution of 55 µm pixel^{-1}.
Standard error bars show variability within each individual microcosm.

3.3.6 MEAN PORE SIZE

Pore size was significantly larger within the loamy sand (0.015 mm^2), followed by the clay loam (0.012 mm^2), with the lowest mean pore size of 0.011 mm^2 found within the sandy loam soil ($F_{2, 2959} = 1099.24$, $P < 0.001$). Harvest period also affected mean pore size with pores significantly larger after fifteen months (0.0141 mm^2) than at time zero (0.0135 mm^2); nine (0.0117 mm^2) and six months (0.0115 mm^2) ($F_{3, 2959} = 411.97$, $P < 0.001$).

At time zero, mean pore size was highest in the loamy sandy soil (0.0152 mm^2) (soil type x harvest interaction, $F_{7, 2959} = 386.21$, $P < 0.001$; Figure 3.22). At the sixth month harvest clay loam microcosms had the highest mean pore size (0.0125 mm^2) followed by the loamy sand (0.0118 mm^2) and the sandy loam (0.0102 mm^2). At the ninth and fifteenth month harvest mean pore size was significantly greater in the loamy sand (0.0131 mm^2 and 0.0189 mm^2 respectively), with the sandy loam and clay loam having a lower mean pore size at the respective harvest periods.

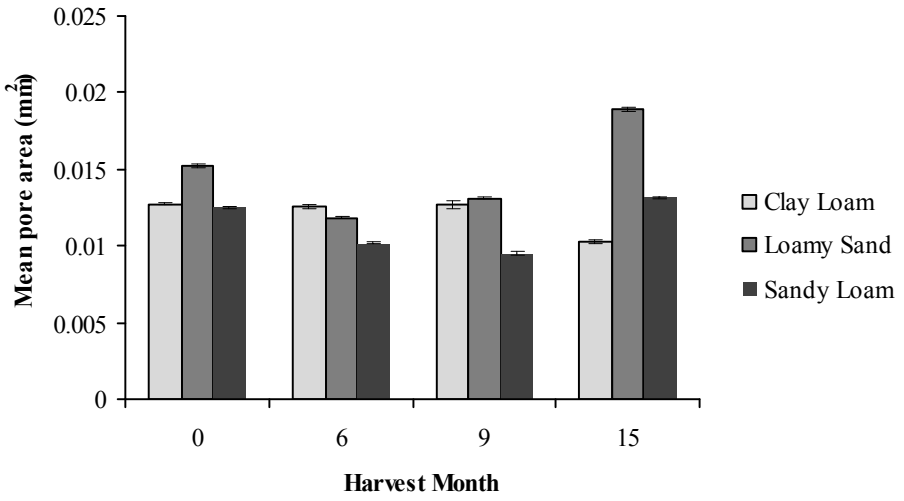


Figure 3.22: Mean pore size for each soil type over each of the five harvest periods for the 270 image slice stack.
Standard error bars show variability within each individual microcosm.

3.3.7 CIRCULARITY

Circularity was highest in the clay loam soil (0.854), followed by the sandy loam (0.815) and then loamy sand (0.790) (soil type effect, $F_{2,2959} = 6870.07$, $P < 0.001$; Figure 3.23). Furthermore circularity was lowest at time zero (harvest effect, $F_{3, 2959} = 933.06$, $P < 0.001$). Over the investigation, circularity increased within the sandy loam from the initial (time zero) scan to the fifteenth month (harvest soil type x harvest interaction, $F_{7, 2959} = 284.24$, $P < 0.001$). Circularity values within the clay loam soil,

did peak at the sixth month harvest (0.874) and with the sandy loam circularity peaking at later harvest periods (0.824 and 0.825 at the ninth and fifteenth months respectively). A summary of all image analysis data for soil type and harvest is given in Table 3.2.

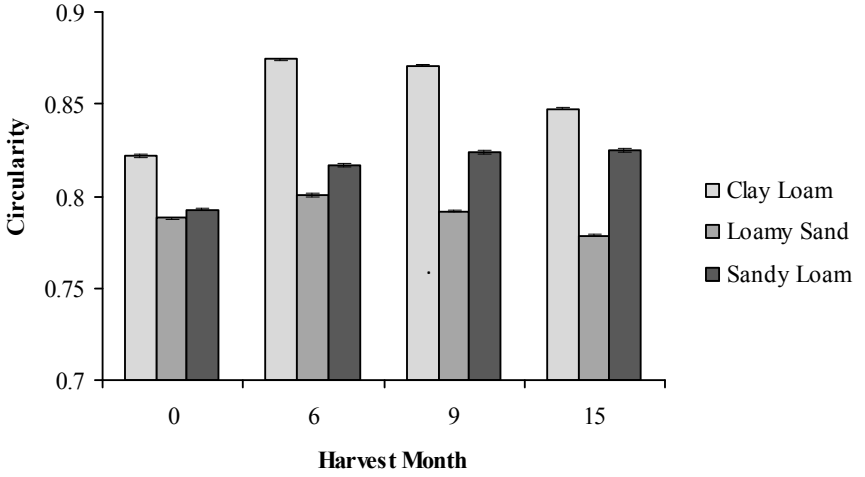


Figure 3.23: Mean circularity of each soil type over the five harvest periods for the 270 slice image stack.
Standard error bars show variability within each individual microcosm.

Table 3.2: Summary data for porosity, mean pore area, circularity and perimeter with soil type and harvest.
Standard error shows variability within each individual microcosm.

Soil Type	Porosity (%)	Mean Pore Area (mm^2)	Circularity
Clay Loam	8.98 ± 0.13	0.0121 ± 0.000073	0.854 ± 0.00076
Loamy Sand	15.19 ± 0.052	0.0148 ± 0.000097	0.790 ± 0.00042
Sandy Loam	13.63 ± 0.060	0.0114 ± 0.000062	0.815 ± 0.00055

Harvest	Porosity (%)	Mean Pore Area (mm^2)	Circularity
Time Zero	11.52 ± 0.035	0.0135 ± 0.000067	0.801 ± 0.00067
6 Months	14.90 ± 0.199	0.0115 ± 0.000067	0.831 ± 0.00012
9 Months	11.62 ± 0.054	0.0117 ± 0.000095	0.829 ± 0.00012
15 Months	10.95± 0.086	0.0141 ± 0.00014	0.817 ± 0.00011

3.3.8 BIOLOGICAL SOIL IMAGING

The mean size of dye stained areas within each image was generally highest in the clay loam soil (particularly at 38 mm from the soil surface) and lowest within the loamy sand at 60 mm depth, suggesting fungal presence was influenced by soil type and depth (soil type x depth interaction, $F_{6,17} = 2.99$, $P = 0.035$). No significant differences in mean size of stained biologically active areas with soil type ($F_{2,17} = 1.50$, $P = 0.251$; Table 3.3) or depth were observed ($F_{2,17} = 0.40$, $P = 0.756$).

Table 3.3: Total percentage of dye stained area and mean size of stained areas within images taken following staining of microbiological components.
Mean values ± standard error.

Soil Type	Total percentage of dye stained area (%)	Mean size of dye stained areas (log mm^2)
Clay Loam	4.913 ± 1.437	0.715 ± 0.043
Loamy Sand	4.036 ± 0.8251	0.641 ± 0.025
Sandy Loam	4.222 ± 0.7055	0.690 ± 0.018

At the top of the microcosms (12 mm from the soil surface) the distance between the centre of biologically active points (nearest neighbour distance) was larger than that deeper within the microcosm (i.e. 44 and 60 mm depth) as seen in Figure 3.24 ($F_{2,20} = 3.45$, $P = 0.038$).

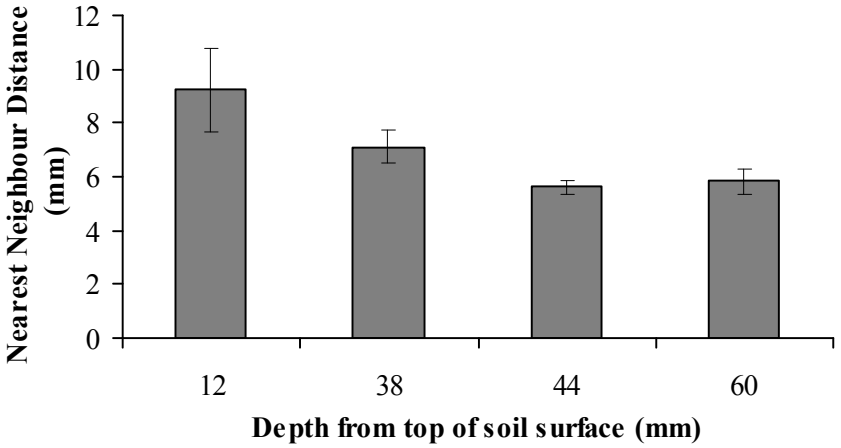


Figure 3.24: Effect of soil depth within the microcosm on the nearest neighbour distance of biologically active areas.
NB: Error bars on graph highlight standard error.

It is clear from Figure 3.25 that the ratio of biologically active pores to non-stained pores within the clay loam soil was greater than that of the sandy loam and clay loam indicating that the clay loam was more microbiologically active. This corresponds to data that highlighted greater numbers of CFUs in the clay loam and also with pore size distributions where large changes were observed after the initial settling period.

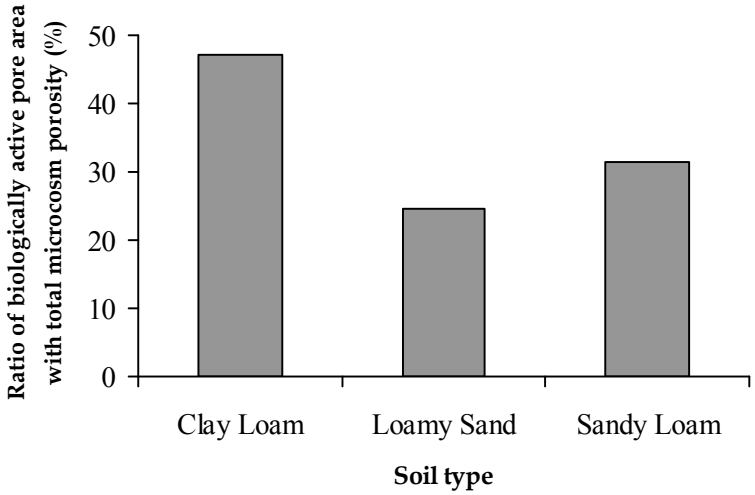


Figure 3.25: Ratio of biologically active dye stained pores to non-stained pores within each soil type at the fifteenth month harvest.

3.3.9 LINKING SOIL PHYSICAL PROPERTIES WITH SOIL TYPE OVER HARVEST PERIODS

3.3.9.1 Relationships between microbial populations and soil physical measurements

Using X-ray μCT it was observed that culturable bacteria influenced pore shape i.e. circularity (Figure 3.26A) within the loamy sand; pore circularity increased with declining bacterial numbers. Furthermore total porosity was found to increase with increasing bacterial numbers (Figure 3.26B). A similar pattern was observed, but this time for fungal numbers, within the sandy loam; with pore circularity decreasing with increasing fungal counts (Figure 3.27A) and porosity increasing with increasing fungal numbers (Figure 3.27B). Such patterns within both soil types provide evidence to suggest that microbial counts within a soil will increase soil porosity and pore morphology.

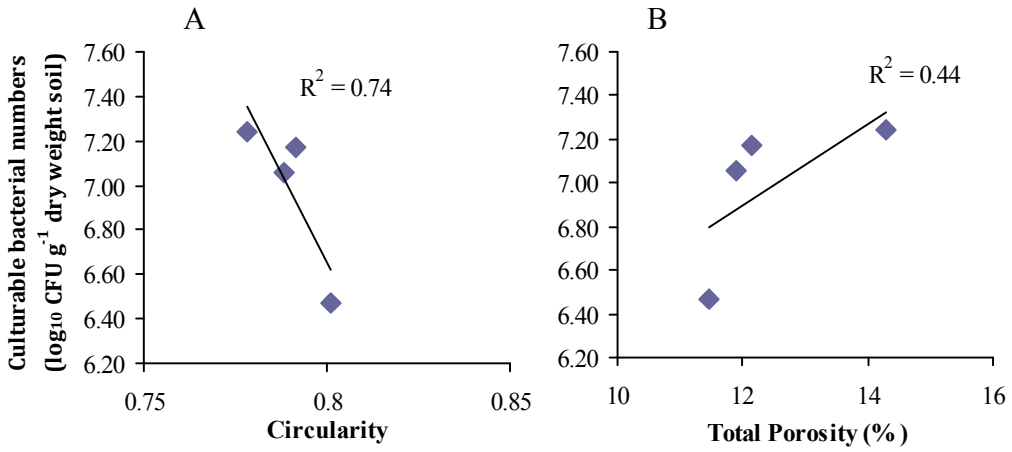


Figure 3.26: Relationship between culturable bacterial numbers and A) pore circularity (Regression, P = 0.027) and B) total porosity (Regression, P = 0.04) within the loamy sand microcosms.

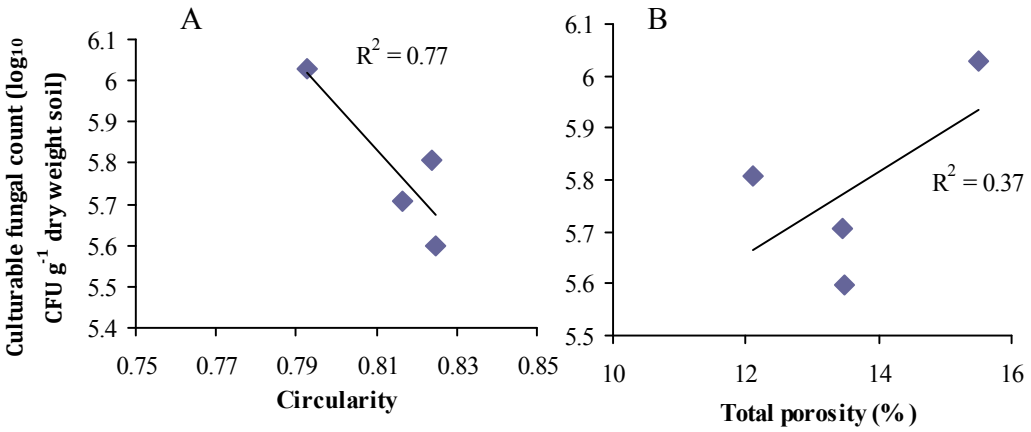


Figure 3.27: Regression between A) total porosity and culturable bacterial numbers within the loam sand microcosms (P = 0.04); B) total porosity and culturable fungal numbers within the sandy loam microcosm (P = 0.03) within the large sampling areas.

Despite the lack of significant relationships between culturable bacterial and fungal numbers on soil structural features within the clay loam microcosms, relationships were observed between total substrate utilisation of Biolog plates and pore area (and hence also with pore perimeter whose results are not shown in detail here). This relationship with total utilisation and mean pore area (and perimeter) was observed over all soil types (Figure 3.28) with mean pore area increasing as total substrate utilisation increased.

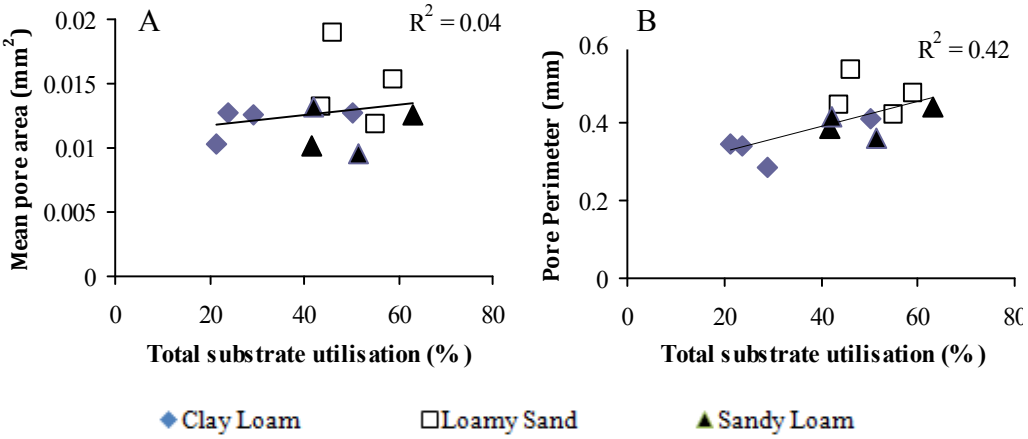


Figure 3.28: Significant regression of total utilisation against A) mean pore area (P = 0.006) and B) pore perimeter (P < 0.001) within the small sampling area.

3.4 DISCUSSION

Soil structural properties were significantly modified due to the presence of microbial communities within the microcosms, with the effect dependent on the soil texture over the course of the investigation. Development of soil structure was observed within all microcosms, after an initial settling of the soil within the microcosms (similar to that observed in the field after tillage by Leij, Ghezzehei and Or, 2002).

3.4.1 CULTURABLE MICROBIAL COUNTS AND METABOLIC COMMUNITIES.

Over the course of the investigation, the clay loam soil had significantly higher culturable numbers for fungi, with bacterial numbers higher than the loamy sand. This may be because clay loam contained the highest percentage of organic matter resulting in more substrates for microbial communities to utilise. These findings agree with Chiarini *et al. (*1998) who discovered that with decreasing particle size, in addition to increasing silt content (Table 2.1), bacterial numbers increase. The results presented

here agree with previous research where soil texture influences microbial compositions, at the level of soil bacterial communities (Chiarini *et al.*, 1998), at the genus level (Latour *et al*., 1996) and at the intraspecific level (Hartmann, Giraud and Catroux 1998; Dalmastri *et al*., 1999).

Despite clay loam having the highest numbers of culturable fungi and the second highest culturable bacterial numbers, the total substrate utilisation within the microcosm was significantly lower compared to the other soil types. Such controls on microbial biomass and activity within soils by soil type were demonstrated by Groffman *et al. (*1996). The reduced activity of microbes within the clay soils maybe due to the smaller soil particles (i.e. clay particles < 2 μm in size), which could in turn clog pore throats and small pores within the soil, leading to a reduction in pore connectivity (Pitty, 1979; Tisdall and Oades, 1982). As a result, nutrient cycling, water and gaseous movement throughout the soil would be restricted reducing the microbial activity within the soil. The effect of soil structure on bacterial metabolic functioning (Biolog) was highlighted by Fang *et al. (*2005) who found average well colour development (AWCD) by communities isolated from a sandy loam soil was significantly lower than that of bacteria from a silt loam and silty clay. Furthermore Girvan *et al*. (2003) also highlighted through the use of T-RFLP, DGGE and Biolog microtitre plates, that total and active bacterial communities were influenced by soil texture. In the current investigation the numbers of culturable fungi and bacteria were highest at the first month harvest, when microbes would have still been adjusting to the soil microcosms and while substrate availability was highest. At the sixth month, culturable numbers declined suggesting the microbes within each microcosm had

reached an equilibrium, i.e. become associated with the surrounding soil environment and substrate availability (Tate, 2000). At the ninth month, fungal and bacterial numbers rose before declining again at fifteen months. This flush of activity at the nine month harvest maybe the result of additional substrates being released into the soil through the decomposition of organisms associated with the lack of nutrients at the sixth month harvest period. Substrate availability is an important factor in changes to microbial activity and communities (Griffiths *et al*., 1999), in addition to the increase in macro-aggregates formation (Denef *et al.,* 2002). Bruneau *et al. (*2005) also observed that bacterial distributions within a scale of less than 1 mm was controlled by fresh faunal excrement, thus highlighting the importance organic substrates have on bacterial distributions within the soil.

Bacteria extracted from the loamy sand preferably utilised amino acids, alcohols, amines and carboxylic acids, whereas bacteria from the sandy loam and clay loam soils mainly utilised amides, aromatic chemicals and phosphorylated acids. This suggests that different soil textures, have different microbial communities with specific catabolic capabilities, or alternatively that the same communities are present irrespective of soil type, but they have different metabolic potentials. It is most likely that communities are soil type specific, indeed Groffman *et al. (*1996) highlighted soil type as a controller of microbial biomass and activity (when assessing the impact of different soil types and grass species).

Results from biologically stained soil thin sections after fifteen months reflect data of culturable bacterial and fungal counts. Loamy sand showed the lowest ratio of dye stained pores to non-stained pores, suggesting fewer biologically active areas compared

to the sandy loam and clay loam. This is in agreement with Degens, Sparling and Abbott (1994) who found that coarse textured soils, such as sand, were weakly related to microbial biomass and products. Furthermore, the biologically stained soil thin sections highlighted that there were larger distances between biological areas at the top of the soil microcosms than with depth. This maybe due to soil microorganisms congregating in areas within, or beside, pore spaces as observed here and by Foster (1988). As pore area tends to be larger and greater towards the top of soil microcosms due to less overburden pressure on the soil (Mohamed and Anita, 1998), microorganisms have larger pore areas to occupy, hence there is greater distance between other biologically active areas. In comparison, at depth, pore space is smaller hence areas of biological activity become more distributed within these smaller pore areas and thus closer together. Thus carroborates the first hypothesis stated in section 1.5. In addition, Nunan *et al. (*2001) suggested bacterial populations grown by self replication lead to the development of colonies in close proximity to each other, similar to that observed here. Hence at the top of the microcosms, where conditions are more favourable for microbial processes (e.g. aerobic and closer to organic matter inputs), larger clumps of biological activity occur near pores and organic substrates resulting in larger distances between these areas. At depth, conditions are less favourable and there is older organic matter (Bruneau *et al*., 2005), hence bacterial population grown by self replication results in colonies of closer proximity, that locate near to any available organic substrate. Such results are similar to those described by Nunan *et al. (*2002), where greater aggregation of bacteria occurred within topsoil than subsoil, where bacterial growth was over a wider area with a short separation distance.

3.4.2 EFFECT OF SOIL TEXTURE ON SOIL STRUCTURE

Through assessment of the μCT images it became apparent that the clay loam microcosms contained a lower total porosity than the other soils. Research by Kiem and Kandeler (1997) found aggregate stability was greatest in sandy soils in comparison to clays. This suggests that pore spaces within sandy soils would be greater than that of the loam and clay soils (as observed in this investigation). This is due to the fine particle nature of the clay loam soil; small clay particles could clog up pores and pore throats resulting in smaller pores than those present within the loamy sand and sandy loam microcosms. Such reduction in porosity of this Worcester series clay loam was also observed in undisturbed field cores taken by Mooney (2002) who found the soil had a total porosity of 24.5 % compared to 31.5 % of a undisturbed sandy loam soil (albeit from a study at a different scale). In the current experiment total porosity was highest within the loamy sand soil which reflects the idea that soil containing a relatively high percentage of sand, i.e. large particle sizes will contain more pore space than that of clay soils (Table 3.2). However, this contradicts results found by Mtambanengwe, Maptumo and Kirchmann (2004) who found porosity decreased with soils of increasing sand quantity.

Porosity followed a similar pattern to that of bacterial and fungal CFU within the soil systems, suggesting an interaction between microbial communities and soil structure. Porosity was lowest within the clay loam soil, where culturable fungal counts were highest and bacterial counts were high, but no significant correlations were found between soil texture and culturable bacterial and fungal numbers. However, when each soil type was assessed over each harvest period, culturable numbers and total substrate

utilisation had an effect on porosity. Within the loamy sand, as bacterial counts increased porosity increased, suggesting bacterial numbers influenced porosity or porosity affected bacterial numbers. Microorganisms are known to produce enzymes and polysaccharides (Tisdall and Oades, 1982; Chenu, 1989; Jastrow and Miller, 1991; Oades, 1993) that bind soil particles forming aggregates and hence increasing porosity. The results of the current study reflect perhaps longer term impacts of bacterial numbers on soil structure development compared to that of previous research by Aşkin *et al. (*no date). In addition, within the sandy loam soil increasing fungal numbers improved soil porosity. This relationship agrees with the widely acknowledged theory that fungal populations within the soil environment improve soil structure through numerous biochemical and biophysical methods (Ritz and Young, 2004; Rillig and Mummey 2006).

With regard to mean pore size, pore area was smallest in the sandy loam soil due to the slightly higher clay content of the soil, particularly in comparison to the loamy sand microcosms, where mean pore size was highest due to the lower clay content and higher sand content. Mean pore size remained lowest within the sandy loam microcosm. Since sand particles are typically the largest and most angular within soil, when these particles aggregate, it leads to the formation of larger pores (see seen in the conceptual model of Boix-Fayos *et al*., 2001). Such variations in mean pore size with soil type have been previous noticed (Thomsen *et al.,* 1999). However it should also be remembered that other factors such as organic matter and land practices will also influence pore sizes.

Pore circularity was highest in the clay loam due to the clay particles (which are small and plate shaped since they are highly weathered (Rowell, 1994)), resulting in the formation of more rounded pores compared to larger angular particles, such as those associated with a coarse sand soil for instance. Furthermore this may also be due to the higher culturable microbe counts and increased microbial activity within these clay loam microcosms. This is less likely however, since a significant negative regression was observed with culturable bacterial and fungal numbers, with circularity within the loamy sand and sandy loam microcosms. The pores within the loamy sandy microcosms had a lower circularity value compared to the sandy loam soil. This may be due to the influence of pore size since the larger the pore, the higher the probability that a pore is elongated or planar, hence the lower the probability that it is round (Mermut, Grevers and de Jong, 1992; Pachepsky, Rawls and Timlin, 2000).

3.4.3 EFFECT OF TIME ON SOIL STRUCTURAL DEVELOPMENT

Soil structure within the microcosms appeared to decline initially due to settling of soil particles after packing (during the inoculation and watering regime). After this period, total porosity increased after nine and fifteen months suggesting an improvement in soil structure. Other soil structure measures such as mean pore area, were higher after fifteen months of incubation than at any other stage signifying an improvement in pore size over time; critical for a good soil structure and microbial processes. Although such soil structural improvements in this investigation were slower than those highlighted in similar studies (e.g. aggregate turnover rates ranging from 4-88 days by Plante, Feng and McGill, (2002) and De Gryze, Six and Merckx (2006)), this study represents the

first known assessment of total porosity and mean pore size over time, particularly within a bare soil system (without the influence of AMF, roots or organic matter).

Pore circularity was generally found to be relatively circular (with values ~ 0.8) over the investigation, with more elongated pores found at the initial time zero and final fifteenth month, compared to the other harvest periods. Such results coincide with pore size data, illustrating that as pores become smaller, pore circularity increases. This would be expected since pore sphericity measurement is a function of size.

3.4.4 EFFECT OF MICROBIAL POPULATIONS ON SOIL STRUCTURE

Increases in soil porosity were correlated with increases in culturable bacterial and fungal counts (thus agreeing with the second hypothesis stated in section 1.5). This is because, as the number of bacteria and fungi increase, activity also increases, resulting in enhanced exudates production from these microorganisms. Exudates are widely acknowledged to improve aggregation and stabilisation within the soil environment, resulting in an increase in porosity within and between aggregates (Tisdall and Oades, 1982; Amellal *et al*., 1998; Czarnes *et al*., 2000).

Other important interactions between total substrate utilisation and mean pore size were observed, with large pores associated with a higher metabolic potential (microbial activity). This once again relates to the importance exudates have on soil structure. As microbial activity increases, the release of these polysaccharides that act as gums and glues will increase resulting in an increase in aggregation and hence resulting in larger pore spaces. The reverse of this could also be true, with large pore area causing an

increase in total substrate utilisation. This is due to the function larger pores have within a soil environment, acting as channels for water carrying nutrients and substrates (for microbes) and for gaseous movement, making these pore sites ideal for microbial functioning and hence higher activity. It was recognised by Nunan *et al. (*2001), with bacteria clumping near pore spaces, where substrate availability, water, air and nutrient flow would be highest. Hence if pores are larger within a soil, this would increase the area available for microbes to inhabit (that are high in resources) leading to an increase in soil activity. Such results therefore highlight the dynamic nature of the soil environment, with individual factors having multiple influences.

3.5 CONCLUSION / SUMMARY

- Soil texture influenced development of soil structure within the microcosms. The loamy sand had the highest porosity and mean pore size of the three soil textures analysed, illustrating that a relatively high percentage of large particles, is beneficial to pore space development.

- Pore shape was influenced by soil texture, with more circular pores associated with the clay loam soil, whose particles tend to be smaller and more rounded.

- Soil texture had a significant effect on culturable microbial communities within the microcosms with the clay loam soil having the highest culturable fungal numbers and second highest bacterial numbers. Although despite this,

microbial activity within this soil was significantly lower compared to the loamy sand and sandy loam soils.

- Soil texture was found to affect the substrate utilisation preferences of microbial communities within specific soil types.

- Culturable microbial counts (bacterial and fungal) were influenced by harvest period. Culturable numbers as expected were highest at the start of the investigation when soil substrate availability was highest. A peak towards the end of the experiment at nine months signified an increase in microbial activity associated with decomposition of microbes within the substrate poor soils. Microbial activity over the course of the experiment (assessed using Biolog microtitre plates) showed a peak in activity at the third month, with a steady decline in activity towards the end of the investigation.

- Soil structure within the microcosms noticeably improved over time after initial soil settling (which decreased porosity within all microcosms at the sixth month harvest). Total porosity almost recovered to that observed initially, thus suggesting soil structural re-development took place over a nine month period (between six and fifteen months) in spite of the reduced microbial activity and numbers associated within this period. Furthermore measurements of mean pore area also highlighted the increase in pore area after the initial decline between time zero and six months.

- Soil structural development (i.e. assessed through measurements of total porosity and mean pore size) was found to be directly influenced by culturable bacterial and fungal numbers in addition to the total microbial activity within the soil, assessed through Biolog microtitre plates. These results highlight the complex nature of soil structure development and suggest that structural development can take place within bare soils detached from the physical and biological impacts plants and their roots introduce. This study therefore illustrates the true importance of microorganisms within the soil environment and particularly on the development of soil structure.

4 EXPERIMENT 2: THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND ROOTS ON THE DEVELOPMENT OF SOIL STRUCTURE.

4.1 INTRODUCTION

Soil microbes significantly contribute to soil ecosystem functions e.g. decomposition, nutrient cycling and the formation and stabilisation of soil structure (section 1.2.2 and 1.2.3). What is not known however is how microbially diverse the soil ecosystem needs to be in order to maintain such functions. Davidson and Grieve (2006) suggest some species within the soil environment have key or strong ecological functions, whereas a loss of other species has no effect. The aim of this investigation was to determine the effect on soil structure of differing levels of microbial diversity, in soil macrocosms containing mycorrhizal and non-mycorrhizal *Plantago lanceolata*. Although roots and arbuscular mycorrhizal fungi (AMF) influence soil structure through their activity (Tisdall and Oades, 1982; Angers and Caron, 1998; Czarnes *et al*., 2000; Read *et al*., 2003; White, 2006), the relative importance to the development and maintenance of soil structure, in relation to differing 'background' microbial diversity has not yet been properly assessed.

Furthermore, previous research regarding the time scale of soil structural development is somewhat limited. Tisdall and Oades' (1982) model regarding the hierarchical development of soil, is still highly regarded. This model suggests the importance of bacteria, fungi and roots as binding and stabilising agents within the soil environment, with their temporal contribution ranging from weeks to years. A recent study by

Feeney *et al. (*2006a) suggests that soil structure and water repellency can be influenced by root and microbial activity extremely quickly. Their investigations show that the number of aggregates > 2000 μm significantly increased over a 30 day period, particularly in rhizospheric soil. Such rapid changes were also similarly observed with water repellency within various soils as a result of increased active fungal populations. Feeney *et al. (*2006a) also produced images from μCT which suggest that roots and microbes have an impact on soil structure and in particular pore distribution within aggregates after a 30 day incubation period.

This investigation aimed to assess the development and maintenance of soil structure under differing background microbial diversities, using image analysis to assess soil structure, combined with traditional techniques such as aggregate stability and size distribution, in addition to Terminal - Restriction Fragment Length Polymorphorism (T-RFLP) analysis of the microbiota. Throughout this investigation differing soil environments were assessed including bare soil and planted systems with and without AMF. The hypotheses tested during this experiment relate to hypotheses one, two and three stated in section 1.5.

4.2 MATERIALS AND METHODS

4.2.1 MACROCOSM CONSTRUCTION

Air dried sterile Dunnington Heath (sandy loam) soil was packed into plastic columns (17.1 cm length x 7.6 cm internal diameter) to a bulk density of 1.1 g cm^{-3}. Mesh 400

μm (Cadisch Precision Meshes Ltd, London, UK) was glued to the base of each column to allow capillary rise of water from base trays to prevent cross contamination of soil columns through watering.

Macrocosms were packed as shown in Figure 4.1. Each column was packed in quarters using the appropriate quantity of soil per quarter to create a bulk density within the whole column of 1.1 g cm^{-3}. This soil was placed into the column and gently tapped down using a packing disc; the soil surface was then disturbed using a spatula before addition of the next quantity of soil in order to prevent packing layers being formed. Between the 3rd and 4th quarter of soil packed into the macrocosm, a layer of 19 g AMF inoculum (PlantWorks Ltd, Sittingbourne, Kent, UK) was placed into the column. This inoculum was added to the two treatments containing AMF, whilst the four non-mycorrhizal treatments contained the same quantity of sterilised inoculum. Inoculum was sterilised in an autoclave (121 ^{o}C and 15 PSI) twice before addition to the macrocosms. The AMF inoculum used in this investigation contained five different endomycorrhizal fungi (*Glomus intraradices*, *G. microagregatum*, *G. mosseae*, *G. geosporum* and *G. claroides*).

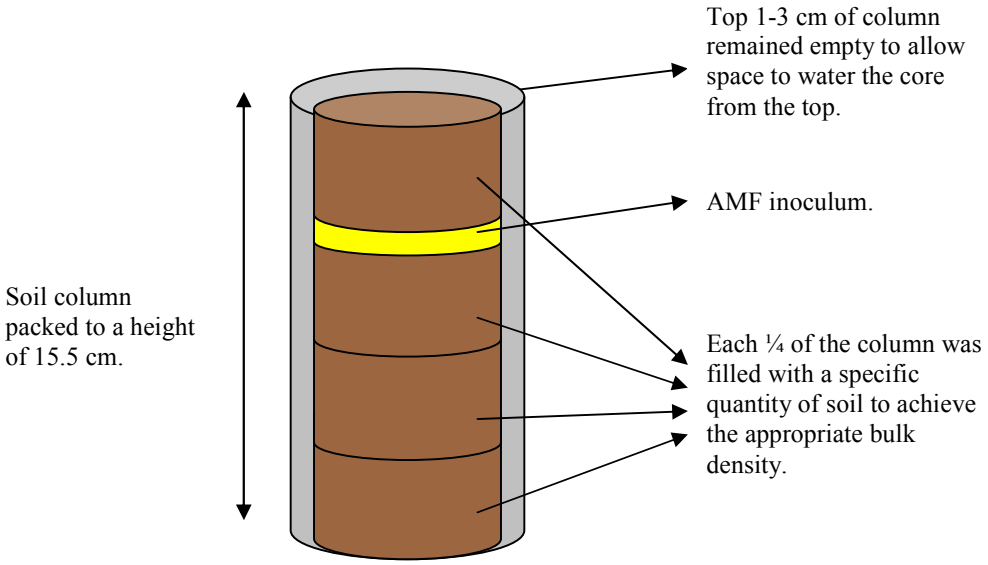


Figure 4.1: Column design for Experiment 2.

4.2.2 SOIL INOCULATION

Experimental macrocosms were inoculated using a 10^1 (low) and a 10^6 (high) dilution (Salonius, 1981; Griffiths *et al.,* 2001) as described in section 2.1.2. Prior to the start of the experiment, seeds of *P. lanceolata* were sown into Dunnington Heath topsoil mixed with sand to improve drainage and allowed to germinate. Seedlings were transplanted into the experimental columns at the one-true leaf stage of growth at a density of one plant per column (Figure 4.2).

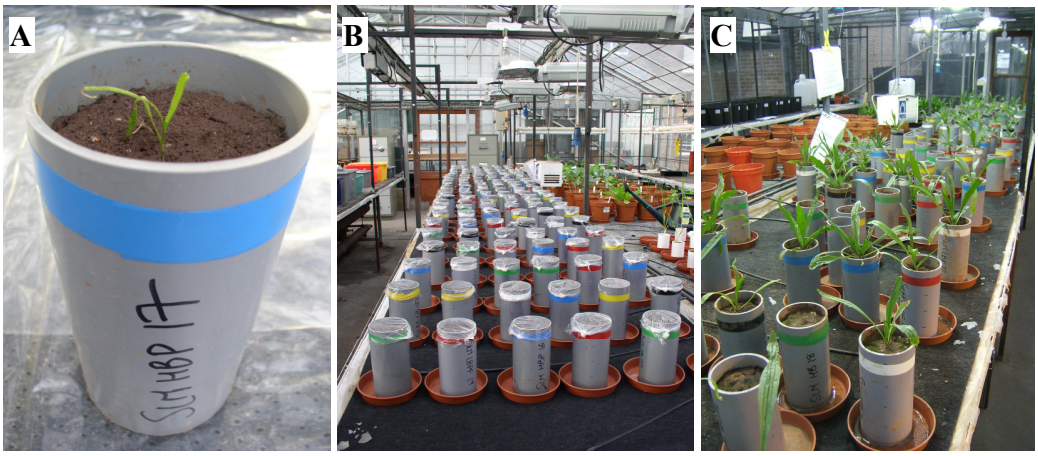


Figure 4.2: Example of a) soil column with *P. lanceolata* and b) and c) the glasshouse containing the experimental columns.

4.2.3 EXPERIMENTAL TREATMENTS

The soil macrocosms were allocated to one of six different treatments; (i) bare soil with 10^1 dilution; (ii) bare soil with 10^6 dilution; (iii) soil planted with *P. lanceolata* at 10^1 dilution; (iv) soil planted with *P. lanceolata* at 10^6 dilution; (v) soil planted with *P. lanceolata* and AMF inoculum at 10^1 dilution; (vi) soil planted with *P. lanceolata* and AMF inoculum at 10^6 dilution. Three replicate columns were destructively harvested per treatment at the first two sampling periods, with four replicate columns harvested at the penultimate and final sampling points. A total of 84 columns were harvested during the experiment. Macrocosms were incubated in a glasshouse at 20–30 ^{o}C, with supplementary lighting to give a 16-hour day throughout the whole investigation (Figure 4.2). Soil columns were maintained at field capacity by watering with sterile (autoclaved) deionised water; the quantity added was determined by weight.

4.2.4 *HARVEST REGIME AND MEASUREMENTS*

Soil macrocosms were destructively harvested at one, three, five and seven months after inoculation and plant transplanting for microbial and structural assessment as described in section 2.1.3 and Table 1.3. The final harvest was undertaken at seven months, as the macrocosms were becoming root bound at this stage (Figure 4.3). Additional measurements solely used in this experiment alone have been described below.

Figure 4.3: Examples of soil removed from macrocosms at the seventh month harvest from the a) bare soil; b) planted non-AMF and c) planted AMF treatment plus the 10^{1} soil dilution.

4.2.5 *AGGREGATE WATER REPELLENCY*

The most appropriate methodology for assessing an aggregate's hydraulic properties and water repellency is by use of a miniaturised infiltrometer (Leeds-Harrison, Youngs and Uddin, 1994; Hallett and Young, 1999). The method described below allows the

hydraulic conductivity, sorptivity and water repellency of individual dry aggregates to be measured. Hydraulic conductivity describes the ease with which water can move through pore spaces or fractures, whereas sorptivity is the capacity of soil to absorb or desorb water through capillarity (Philip, 1957).

The hydrophobicity of an aggregate is determined through measurement of the sorptivity of ethanol and water. Since ethanol has a non-polar nature, the contact angle with the hydrophic surfaces provides a transport measurement that is not influenced by repellency. An index of water repellency can be evaluated by comparing the sorptivity values of water and ethanol for a particular aggregate which allows the extent to which water sorptivity is altered by soil particle coatings to be described. Such methodology, allows identification of the changes in pore structure associated with roots, in addition to changes in repellency caused by roots and microbial exudates (Hallett, Gordon and Bengough, 2003).

4.2.5.1 Experimental procedure – hydraulic conductivity

Soil removed from the macrocosms was air dried for 7-14 days and sieved to 2-5 mm in size. These 2-5 mm aggregates were oven dried at 40 ^{o}C for 24 hours to stimulate an extreme drying event (Hallett and Young, 1999). Twelve replicates per treatment were assessed, with 4 replicates taken from each macrocosm at the first and third month harvest and 3 replicates taken from each macrocosm at the fifth and seventh month period.

The infiltration device was constructed with the capillary tubing and glass tubing (internal diameter 3.5 mm) attached and sealed with sealant (Figure 4.4 and 4.5). A 200 µl pipette tip was placed onto the end of the infiltration device and sealed to the tubing to prevent air leaks. The device was fixed in a vertical position to allow water to flow within the tubes. A water reservoir was set up in a small plastic bottle (100 ml), with the glass tubing placed inside (but not touching, the base and sides of the bottle). This reservoir was placed onto a balance (GF-200 AND balance, California, USA) to allow infiltrated water to be monitored by connecting the balance with to a laptop and recording the change in reservoir weight every two seconds (RsCom v2.43, WinCT Software, AND, California, USA).

A dried aggregate was placed on the dry surface of a scissor jack before starting the computer log. The stage was raised gently so the tip of the pipette touched the aggregate (Figure 4.5). The change in weight of the reservoir was then monitored over a 2 minute period or until the aggregate had become saturated. In order to assess the hydraulic conductivity, the sorptivity of water flowing into aggregates at five different heads of water were measured (i.e. 0, -10, -20, -30 and -40 mm).

4.2.5.2 Experimental procedure – water repellency

Once the hydraulic conductivity measurements were complete, water repellency measurements were taken. For this assessment the infiltration tip was placed at a hydraulic head of -20 mm and the reservoir filled with water. A dried aggregate's uptake of water was once again monitored over a 2 minute period or until the aggregate

had become saturated. Once the analysis was completed the aggregate was dried for at least 24 hours at 40 ^{o}C before re-analysis with an ethanol solution.

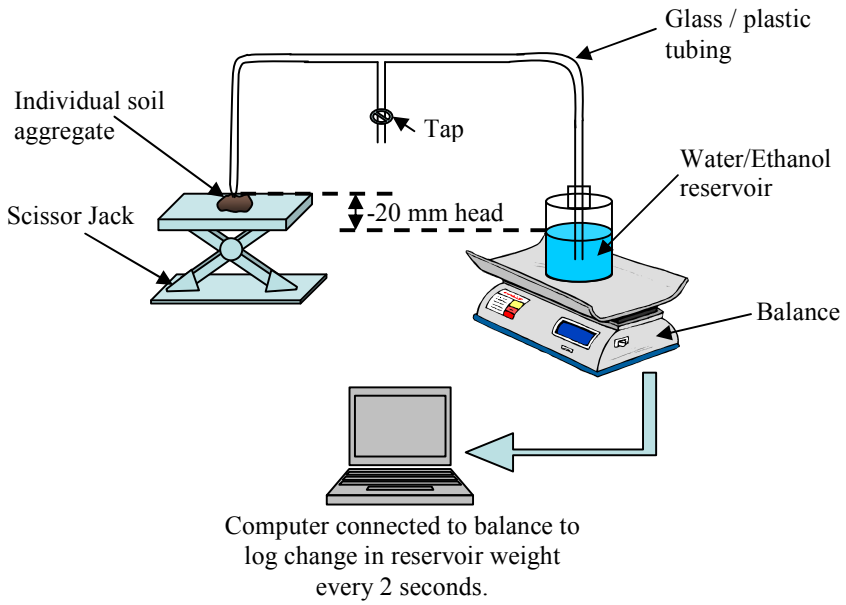


Figure 4.4: Diagrammatic representation of the instrumentation required for testing water repellency of an individual aggregate.

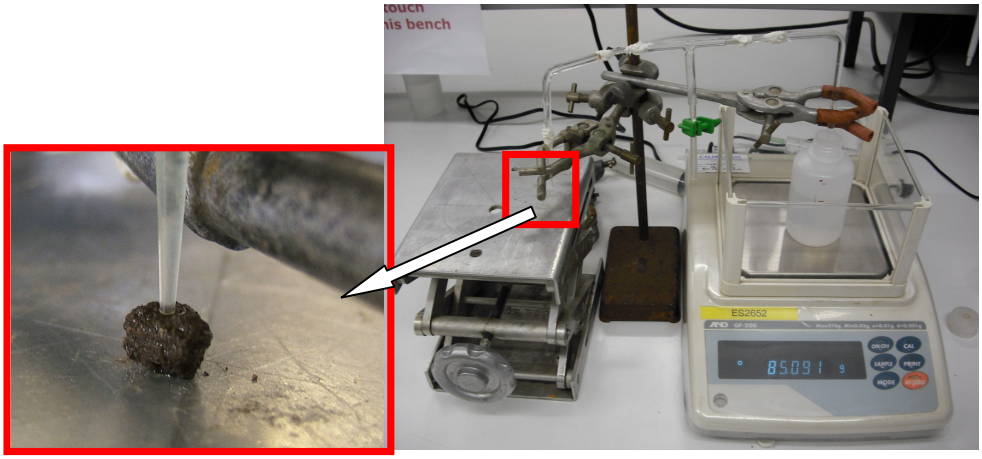


Figure 4.5: Experimental set-up for testing water repellency of an individual aggregate.

Hydraulic conductivity (K) of the aggregate is evaluated from the slope when the steady water flow (Q) is plotted against the different pressure heads (h). Once the hydraulic conductivity of the samples is determined the sorptivity of the aggregate is evaluated from Equation 4.1

$$Q = \frac{\left(4brS_o^2 \right)}{f} = 4rKh \quad \text{(Eq. 4.1))}$$

Equation 4.1: Hydraulic conductivity of individual aggregates Leeds-Harrison and Young, 1997)

Where: Q = Steady state flow ($mm^3\ s^{-1}$); b = A parameter that depends on the soil-water diffusivity function (in the range of $0.5 \leq b \leq \pi/4$) with 0.55 being an 'average' value; r = Radius of the infiltrometer tip (mm); f = Fillable air-porosity (g); h = Pressure head (mm) K = is evaluated from the slope of a plot of Q against h. (mm s^{-1}); S = Sorptivity (mm $s^{-1/2}$).

According to Hallett and Young (1999) for non-repellent soils, the sorptivity of an ethanol to water solution, S_E, is related to the sorptivity of pure water, S_W as seen in Equation 4.2:

$$S_w = \left[\frac{\left(\frac{\mu_E}{\gamma_E} \right)^{\frac{1}{2}}}{\left(\frac{\mu_W}{\gamma_W} \right)^{\frac{1}{2}}} \right] S_E \quad \text{(Eq. 4.2)}$$

Equation 4.2: Sorptivity of ethanol to water solutions into individual aggregates

Where: μ_E = Viscosity of 95 % ethanol at 20 ^oC (0.0012 N s m^{-2}); γ_E = Surface tension of 95 % ethanol at 20 ^oC (0.023 N m^{-1}); μ_W = Viscosity of the water at 20 ^oC (0.0010 N s m^{-2}); γ_W = Surface tension of water at 20 ^oC (0.073 N m^{-1})

Hence the equation is simplified as seen in Equation 4.3 to calculate the repellency index (Equation 4.4) where R = 1.0 signifies a totally non-repellent soil. Furthermore, Tillman *et al. (*1989) also suggested that a soil with $S_E < S_W$ (R<1.95) is non-repellent.

$$S_w = 1.95\ S_E \qquad \text{(Eq. 4.3)}$$

Equation 4.3: Simplified sorptivity of ethanol to water solutions

$$R = 1.95\ (S_E/S_W) \qquad \text{(Eq. 4.4)}$$

Equation 4.4: Repellency index (Hallett and Young, 1999)

4.2.6 QUANTIFICATION OF SOIL STRUCTURE USING X-RAY CT

In addition to invasive techniques, soil structure was assessed non-destructively by X-ray CT tomography using an X-TEK Venlo H series, high resolution X-ray CT scanner (H 350/225 CT; X-TEK, Tring, Hertfordshire, UK). At each harvest period the same two columns, taken from each dilution and treatment, were placed on the rotating sample plate inside the CT apparatus and scanned. The same columns from each treatment were scanned at each harvest point to allow for consistency and reduce any factors causing variation over the incubation periods. Each macrocosm was scanned at 175 kV and 3 mÅ. Exposure time was 90 ms with the samples placed 145 mm away from the detector (Figure 2.5). Copper primary filters (2 mm thick) were used in addition to a 4 mm copper plate on the detector to adjust for beam hardening. Soil columns were scanned at various depths throughout the macrocosms to allow assessment of the soil pore system at various depths. Macrocosms were scanned at a depth of 35, 75 and 115 mm from the top of the soil surface, during the initial first and third harvest periods, with an additional three more depths at 55, 95 and 135 mm from the top of the soil surface assessed at the fifth and seventh month harvest.

4.2.7 IMAGE ANALYSIS

Images obtained using the CT scanner were processed and assessed using AnalySIS® (Soft Imaging Systems (SIS), Münster, Germany) to isolate pore spaces (Figure 4.6). Image resolution was 65.4 μm $pixel^{-1}$. Initial images were cropped to 52.97 x 50.69 mm (810 x 775 pixels), to remove the macrocosm from the image, in addition to boundary effects such as cracks which ran down the edges of the macrocosm.

Filtering was performed to improve quantification of pore features (Figure 4.6) using: 1) An optimise contrast function, providing a maximisation of the contrast, allowing contrast enhancement; 2) a median filter which smoothed the image; 3) a lowpass filter that acted as a noise reduction filter; 4) a sharpen filter, that emphasises detail and is used after noise reduction to reduce the influence of artefacts. Binarisation of the images was undertaken using a modified auto-threshold (where the overflow value was set as 48 %), since default settings did not satisfactorily separate solid from the pore phase. No binary filters were applied to these images since no improvement to the previously acquired image was observed.

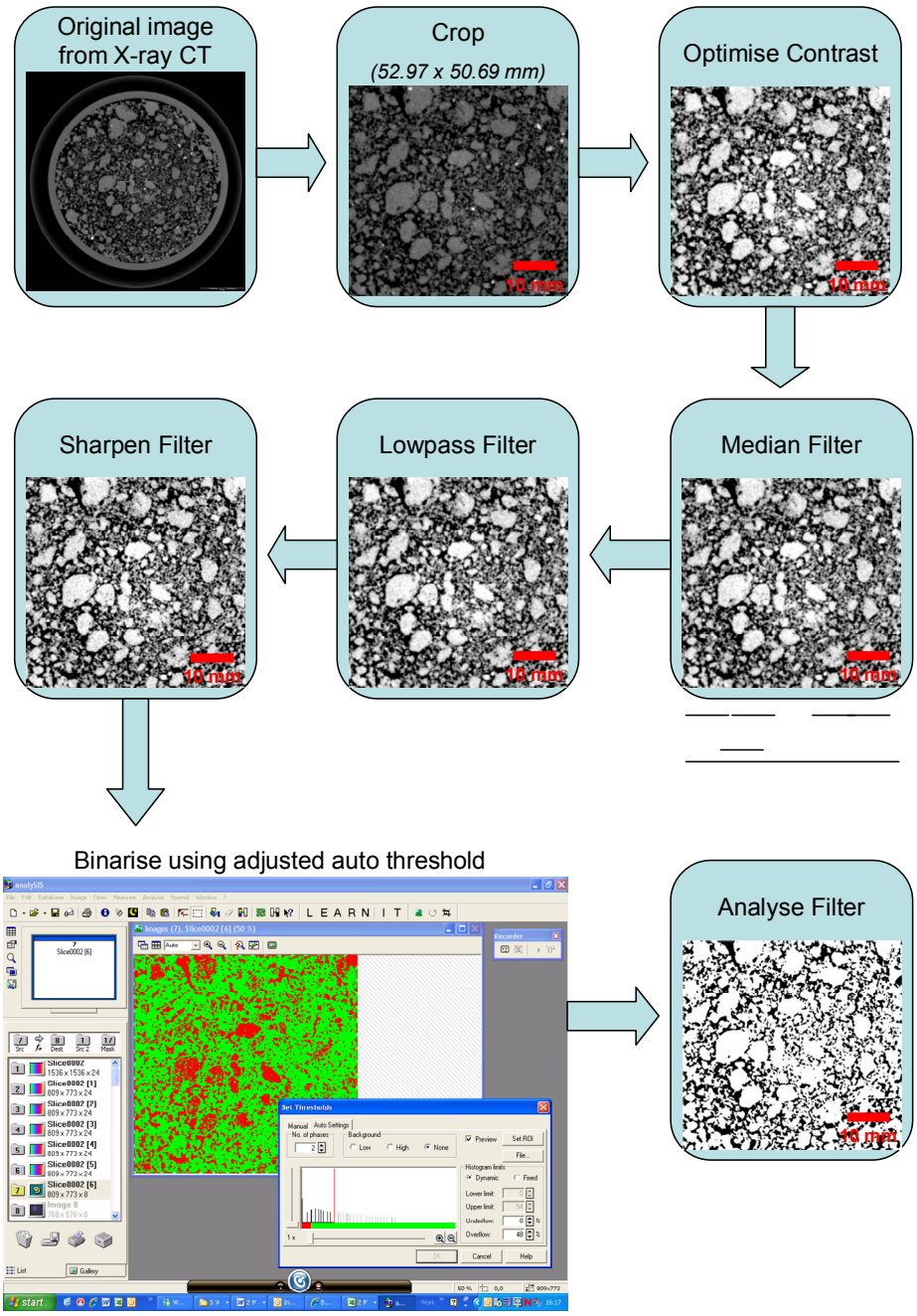


Figure 4.6: Image analysis sequences.
Note: Pore space is indicated in black, with soil represented in white.

Morphological analysis was performed on all images using AnalySIS®, this included the following pore measurements; porosity (total percentage pore area of the sample); mean pore area (average pore size of the pores within the sample); pore perimeter (described as the perimeter of a pore that can determine the roughness (Atkinson, 2008)); sphericity (a measurement of pore's circularity, defined from 1 = spherical to 0 = elongated and flat) and nearest neighbour distance (an assessment of the average distance between pores from centre to centre).

4.2.8 STATISTICS

Analysis of variance (ANOVA) was conducted using soil dilution (10^1 and 10^6), treatment (here defined as either bare soil or planted with and without mycorrhizal fungi) and harvest time as factors. Data were transformed where appropriate (square root for shoot and root biomass, arcsin for mycorrhizal root length colonisation, $\log_{10}+1$ for X-ray CT measurements (i.e. mean pore size, pore perimeter and nearest neighbour distance)). Repeated measures ANOVA was conducted on all X-ray CT measurements, since the same macrocosms were scanned (at the same depth) at each harvest period.

Biolog microtitre plates were corrected using the absorbance values measured within two hours of microtitre plate inoculation. All substrate wells were then corrected for background colour production by subtracting the value of the control well. Total utilisation of Biolog plates (at each record incubation time: 24, 48, 72, 96 and 120 hours) was analysed using repeated measures ANOVA where soil dilution, treatment

and harvest were factors. Principal component analysis (PCA) was carried out on the background corrected average well colour development values that had undergone a natural log transformation using Genstat 11.1. The covariance matrix was used, with analysis of principal component (PC) scores using repeated measures ANOVA in Genstat. PCA was also carried out on T-RFLP data that had been transformed into relative abundance data from all soil macrocosms at each harvest period. The covariance matrix was used as recommended by various papers (Ramette, 2007; Culman *et al*., 2008) with principal component (PC) scores analysed by ANOVA.

4.3 RESULTS

4.3.1 ORGANIC MATTER

Throughout the experiment organic matter content was highest within the 10^6 treatment (dilution effect, $F_{1,57} = 6.38$, $P = 0.014$). Furthermore organic matter content was influenced by harvest time with organic matter highest at the three month harvest ($F_{3,57} = 219.09$, $P < 0.001$) (Figure 4.7). No differences were observed between the third and fifth harvests, however at the seventh month harvest a significantly lower organic matter content was observed than at the third and fifth months.

Organic matter content was lowest ($F_{2,57} = 27.90$, $P < 0.001$) within the macrocosms containing *P. lanceolata* and AMF, however no differences were observed between the bare soil and the non-AMF planted treatment. Furthermore significant interactions were observed in the bare soil treatment at the 10^6 dilution where organic matter content was

higher than at the 10^1 dilution; across the other treatments soil dilution did not appear to have an impact (dilution x treatment level, $F_{2,57} = 6.37$, $P = 0.003$).

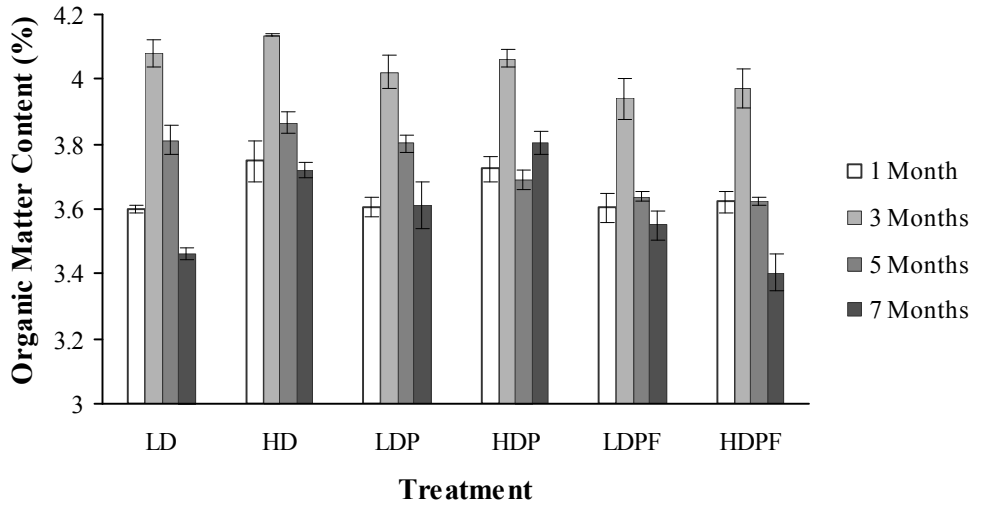


Figure 4.7: Organic matter content of soil within the macrocosms, measured at four harvest periods after inoculation and planting.

ANOVA interactions include treatment x harvest ($F_{6,57} = 5.13$, $P < 0.001$); dilution x treatment x harvest effect ($F_{6,57} = 4.70$, $P < 0.001$). *Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).*

4.3.2 SHOOT AND ROOT BIOMASS

After one month, mycorrhizal plants had lower shoot and root biomass than non-mycorrhizal *P. lanceolata* (Figure 4.8, 4.9, 4.10; Table 4.1). Background soil dilution had no effect in the absence of AMF but in the presence of AMF, the proportional increase in shoot growth from months one to three was most noticeable in the mycorrhizal plants growing within the 10^6 dilution. Stunting of the root growth by AMF was also apparent, but less marked in soils inoculated with the 10^6 soil dilution.

Root growth increased gradually over time in contrast to shoot biomass which did not increase after the third month.

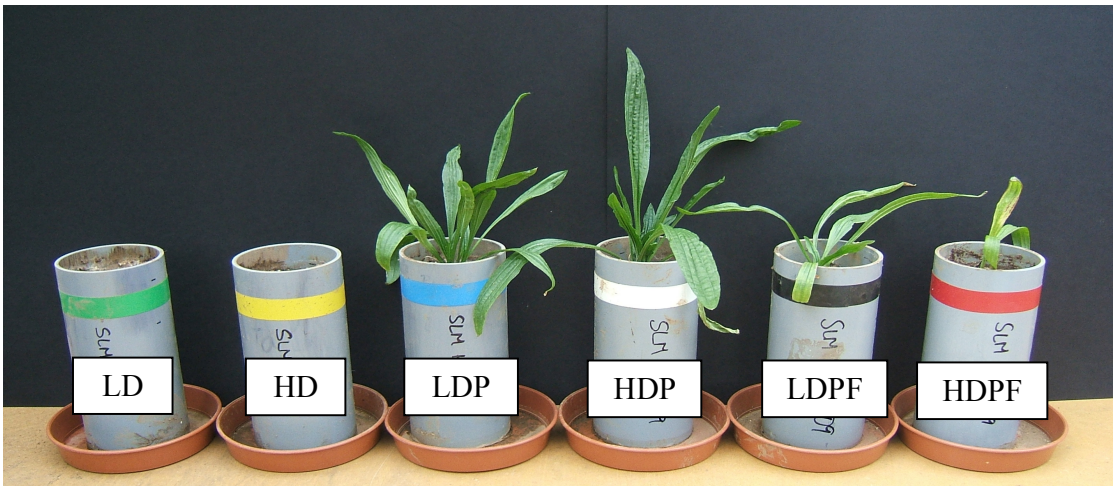


Figure 4.8: All treatments at the one month harvest.
(Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

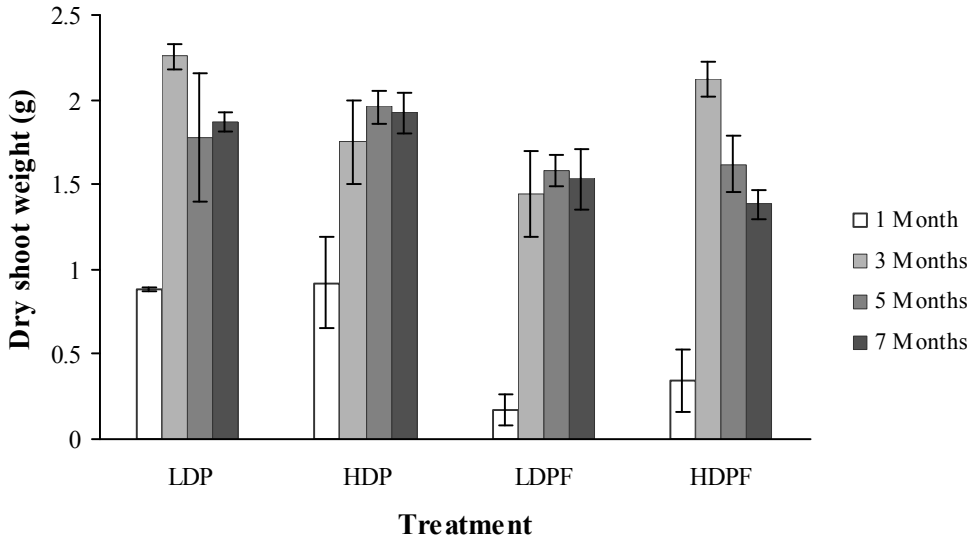


Figure 4.9: Effect of treatment on dry shoot weight of *P. lanceolata*, measured at four harvest periods after inoculation and planting.
Data are means (square root) of replicates ± standard error. (Treatment codes: LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

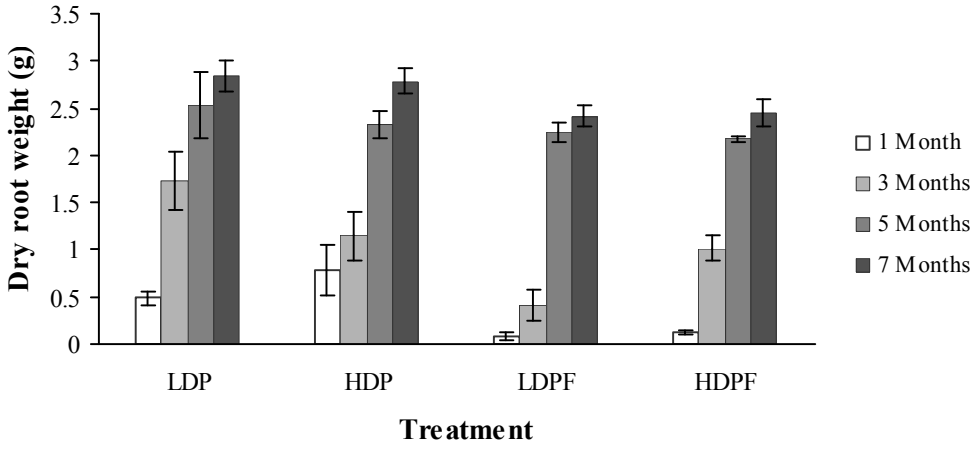


Figure 4.10: Effect of treatment on dry root weight of *P. lanceolata*, measured at four harvest periods after inoculation and planting.

Data are means (square root) of replicates ± standard error. (Treatment codes: LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

Table 4.1: Significant results from ANOVA of shoot and root biomass.

Source of variation	DF	F	P
SHOOT			
Fungi (± AMF)	1	21.20	< 0.001
Harvest	3	49.47	< 0.001
Dilution x Fungi (± AMF) x Harvest	3	3.51	0.024
Residual	*38*		
ROOT			
Fungi (± AMF)	1	30.75	< 0.001
Harvest	3	159.17	< 0.001
Dilution x Fungi (± AMF) x Harvest	3	3.49	0.025
Residual	*37*		

4.3.3 ARBUSCULAR MYCORRHIZAL COLONISATION

Percentage root length colonised was significantly greater in the fifth and seventh months compared to the first and third months (Figure 4.11; $F_{3,16} = 7.24$, $P = 0.003$). Presence of arbuscules and vesicles mirrored that of hyphal colonisation (Figure 4.12).

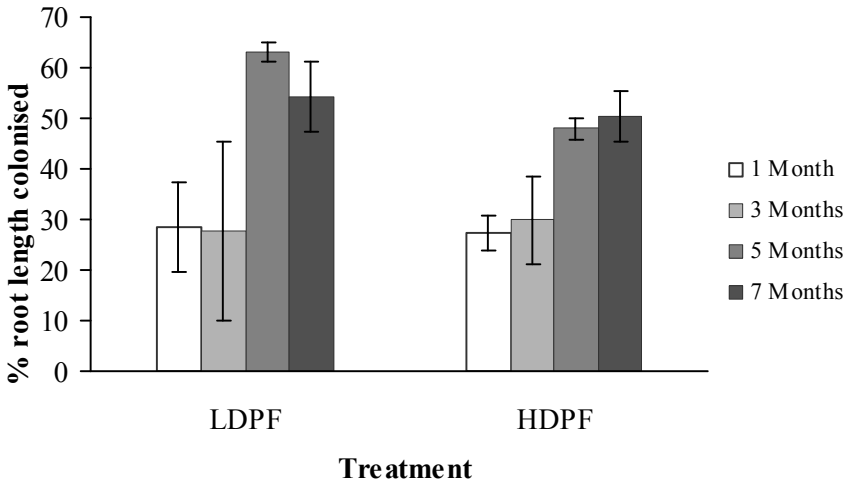


Figure 4.11: Colonisation by arbuscular mycorrhizal fungi of *P. lanceolata*, measured at four harvest periods after inoculation and planting.

Data are means (arcsin transformed) of replicates ± standard errors. (Treatment codes LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment respectively).

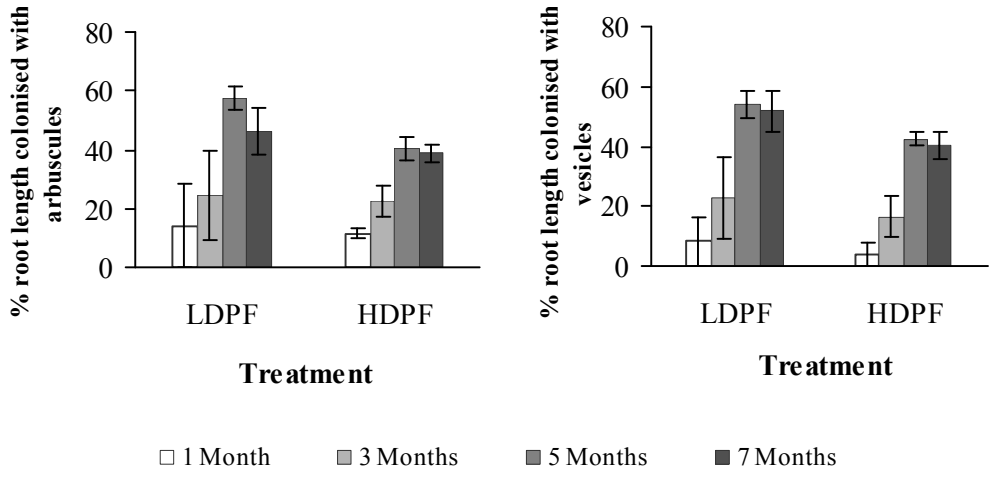


Figure 4.12: Presence of arbuscular mycorrhizal fungi arbuscules and vesicles within *P. lanceolata*, measured at four harvest periods after inoculation and planting.

Harvest effect for arbuscule presence ($F_{3,16} = 9.19$, $P < 0.001$); Harvest effect for vesicle presence ($F_{3,16} = 18.47$, $P < 0.001$). *Data are means (arcsin transformed) of replicates ± standard errors. (Treatment codes LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment respectively).*

4.3.4 *MYCORRHIZAL DEPENDENCY*

The negative values for mycorrhizal dependency (based on shoot biomass) indicate that AMF colonisation severely reduced plant biomass within the first month of growth. At no point did *P. lanceolata* exhibit any positive effect of being mycorrhizal with respect to the measure of dependency (Figure 4.13).

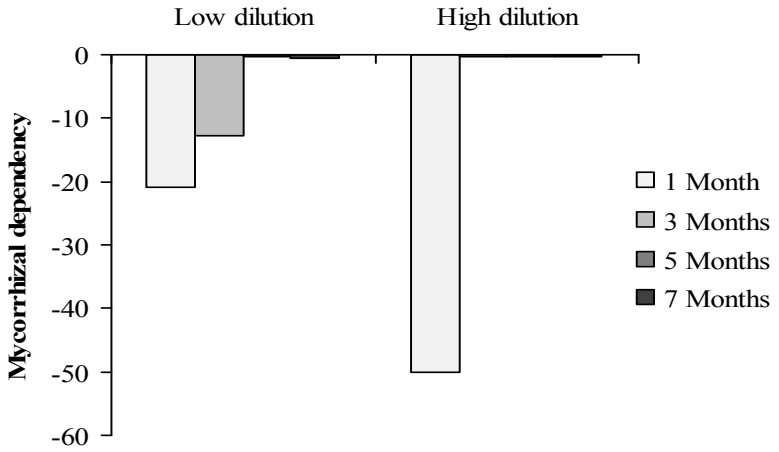


Figure 4.13: Mycorrhizal dependency of *P. lanceolata* over each of the four harvest periods and under both the low (10^1) and high (10^6) dilution levels.

4.3.5 *MICROBIAL BIOMASS*

Mean microbial biomass was 201.4 µg C g^{-1} soil within the 10^1 dilution and 165.0 µg C g^{-1} soil within the 10^6 dilution (dilution effect, $F_{1,40} = 30.44$, $P < 0.001$). In addition, macrocosm treatment had a significant effect on microbial biomass ($F_{2,40} = 153.03$, $P < 0.001$) with biomass increasing from the bare soil treatment (112.0 µg C g^{-1} soil), to the planted treatment +AMF (174.6 µg C g^{-1} soil) and finally to the non-mycorrhizal planted macrocosms (268.1 µg C g^{-1} soil). Microbial biomass was highest after five

months with a mean value of 227.5 µg C g^{-1} soil compared to 120.4 µg C g^{-1} soil in the first harvest (harvest effect, $F_{3,40} = 74.10$, $P < 0.001$).

Microbial biomass was lower in the planted (± AMF) treatments at the 10^6 dilution, compared to the 10^1 dilution (dilution x treatment interaction, $F_{2,40} = 11.65$, $P < 0.001$; Figure 4.14). Furthermore after one month, biomass-C was higher at the 10^6 dilution than at the 10^1. This pattern then reversed in the third month and continued into the fifth month, after which biomass reached an equilibrium in the seventh month.

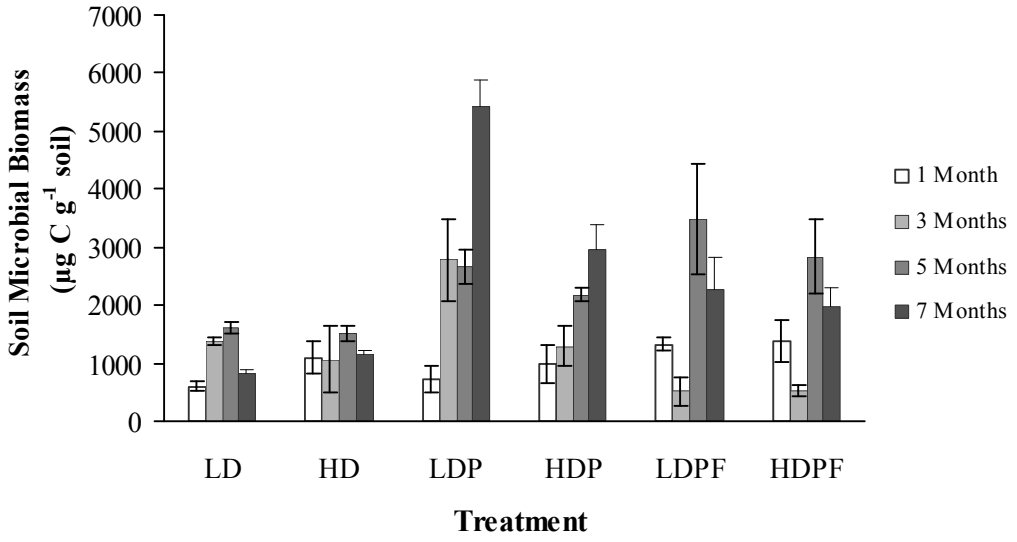


Figure 4.14: Soil microbial biomass-C within the macrocosms, measured at four harvest periods after inoculation and planting.

Dilution x treatment x harvest effect ($F_{6,40} = 11.46$, $P < 0.001$)

Data are means of replicates ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

4.3.6 SOIL MICROBIAL COMMUNITY METABOLIC ANALYSIS

Dilution level, treatment, harvest period and incubation time all had a significant effect on the total utilisation of carbon substrate present in Biolog microtitre plates. A greater

number of substrates were utilised by communities extracted from macrocosms given the 10^1 dilution than from soils amended with the 10^6 dilution (56.21 % and 48.99 % respectively) ($F_{1,57}$ = 87.88, P < 0.001). The bare soil treatment had the lowest total utilisation (45.05 %), followed by the planted non-AMF (52.38 %) and the planted mycorrhizal treatment (60.36 %) (treatment effect, $F_{2,57}$ = 132.54, P < 0.001). Furthermore total utilisation was significantly higher after seventh months (55.21 %) compared than at any other time (~ 52 %), (Figure 4.15a-d; $F_{3,419}$ = 6.32, P < 0.001).

In addition Biolog incubation time had a significant impact on the total utilisation ($F_{4,240}$ = 6345.79, P < 0.001) with total utilisation increasing significantly from 24, 48, 72 and 96 hours from 2.56, 50.63, 65.25, and 71.64 % respectively (Figure 4.15). Total utilisation after 120 hours (72.91 %) remained similar to that observed at 96 hours.

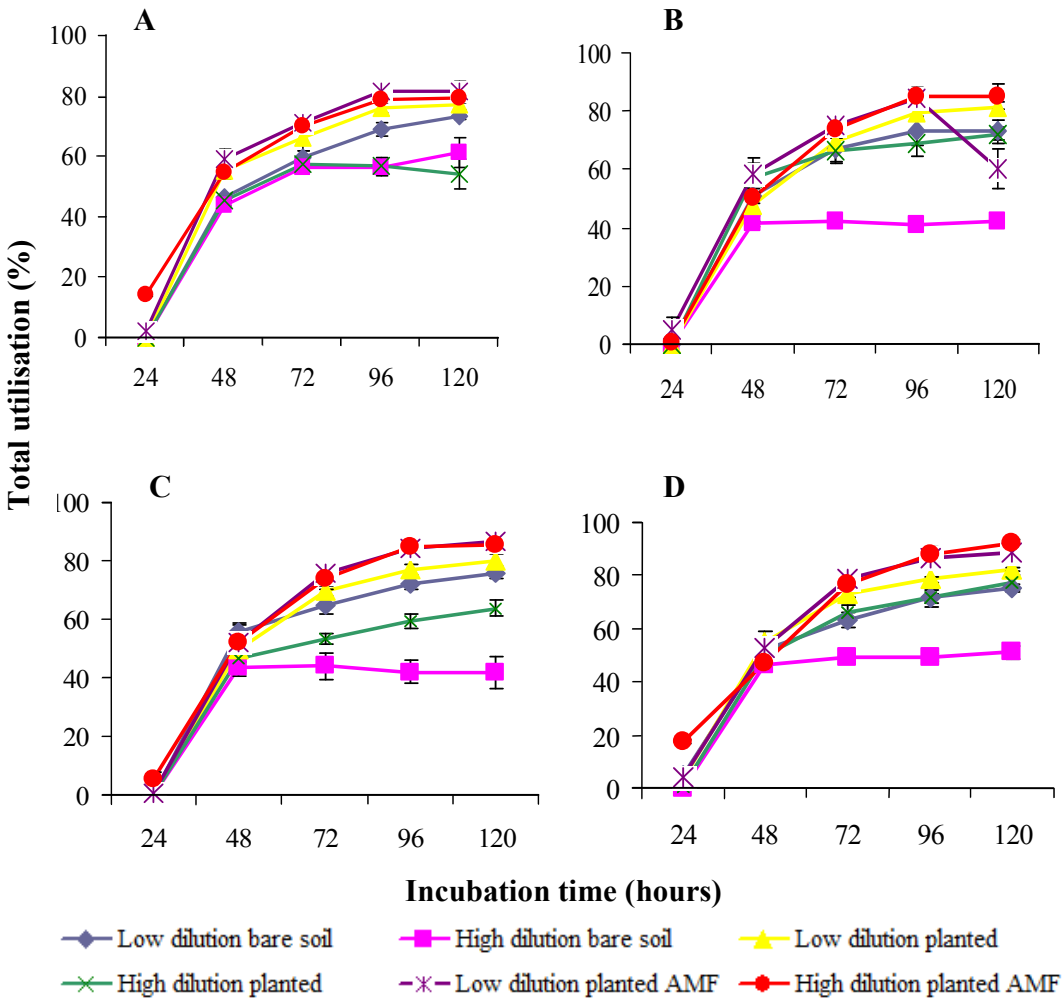


Figure 4.15: Total substrate utilisation for each treatment and dilution level after a) one month, b) three months, c) five months and d) seven months from macrocosms establishment.

Biolog plates were incubated for 120 hours. Data are mean values of replicate macrocosms; vertical bars represent the standard error of the mean.

Further assessment of substrate utilisation was undertaken by measuring the rate of change in total substrate utilisation over the 48-96 hour incubation period, since this was the incubation time period in which most colour development took place. Bacterial communities originating from soils amended with the 10^{1} dilution exhibited a higher

rate of substrate utilisation than those from the 10^6 dilution (Table 4.2). Substrate utilisation rate change was lowest (5.68 %) in the bare soil treatment, and highest within the planted macrocosm with mycorrhizal fungi (15.75 %) compared to the planted non-AMF macrocosm (10.09 %). A dilution x treatment interaction showed the rate of substrate utilisation was lowest within the bare soil amended with the 10^6 dilution and fastest by bacteria from the 10^6 dilution macrocosm containing mycorrhizal fungi and *P. lanceolata* (Table 4.3).

Table 4.2: Results of an ANOVA of the rates of substrate utilisation within the macrocosms at all harvest periods.

Source of Variation	DF	F	P
Dilution	1	46.03	< 0.001
Treatment	2	92.48	< 0.001
Harvest	3	2.56	0.064
Dilution x Treatment	2	30.14	< 0.001
Dilution x Harvest	3	2.44	0.074
Treatment x Harvest	6	5.98	< 0.001
Dilution x Treatment x Harvest	6	1.99	0.082
Residual	*57*		

Table 4.3: Rates and rank order of substrate utilisation between 48-96 hours within the macrocosms at all harvest periods.

Data are means of replicates ±standard error. Values in brackets represent the rank order of utilisation changes.

Treatment	10^1 dilution (%)	10^6 dilution (%)
Bare soil	9.85 ± 0.65 *(4)*	1.50 ± 0.89 *(6)*
Planted – AMF	12.86 ± 0.86 *(2)*	7.33 ± 0.87 *(5)*
Planted + AMF	14.66 ± 1.02 *(2)*	16.84 ±1.11 *(1)*

Principal component analysis (PCA) of background-corrected AWCD values for all 95 carbon sources on the Biolog GN2 microtitre plate was performed on data collected

after 24, 48, 72, 96 and 120 hours incubation. The first principal component (PC1) accounted for 78.61 % of the total variation, with the second component (PC2) accounting for 5.00 % of the variation (Table 4.4). PC1 and PC2 accounted for 83.61 % of the total variation, hence other PCs were negligible and were not incorporated into analysis.

Table 4.4: Results of principal component analysis of background-corrected (ln +1 transformed) AWDC values for all 95 carbon sources in Biolog GN2 microtitre plates for each treatment and background dilution level, at each harvest period.
NB: Latent root or eigenvalues are the sum of the squared factor loads for any given factor and can be thought of as the amount of variance for that factor.

	PC1	PC2
Latent root	2.857	0.182
% Variance	78.61	5.00
Cumulative	78.61	83.61

PC1 and PC2 loadings for each substrate are shown on separate axes (Figure 4.16). All PC1 loadings were negative; therefore the main axis of variation is one where the colour intensity of all substrates decreases or increases in unison. However, PC1 loadings do seem to vary in magnitude with substrate type, with amino acids, carbohydrates, polymers and amines more negative than bromidated chemicals, phosphorylated chemicals and alcohols. Soil dilution, treatment and harvest period had no significant effect on PC1 loadings with substrate guilds, suggesting that variation within groups was greater than between each substrate group (ANOVA on PC1 loadings; $F_{10,84} = 1.86$, $P = 0.062$). This was also true for PC2 loadings which showed greater variation within substrate groups than between ($F_{10,84} = 1.80$, $P = 0.073$). Differentiation occurred between substrates with positive PC2 scores that were utilised,

and substrates with negative PC2 scores that were not utilised. In terms of substrate guilds, carboxylic acids and amides were mainly positive, whereas aromatic chemicals, carbohydrates, esters, phosphorylated chemicals and polymers are negative. Hence, if carboxylic acids and amides were utilised, aromatic chemicals, carbohydrates, esters, phosphorylated chemicals and polymers are not. Amino acid substrates were positive and negative, showing certain individual C sources were preferred over others within guilds.

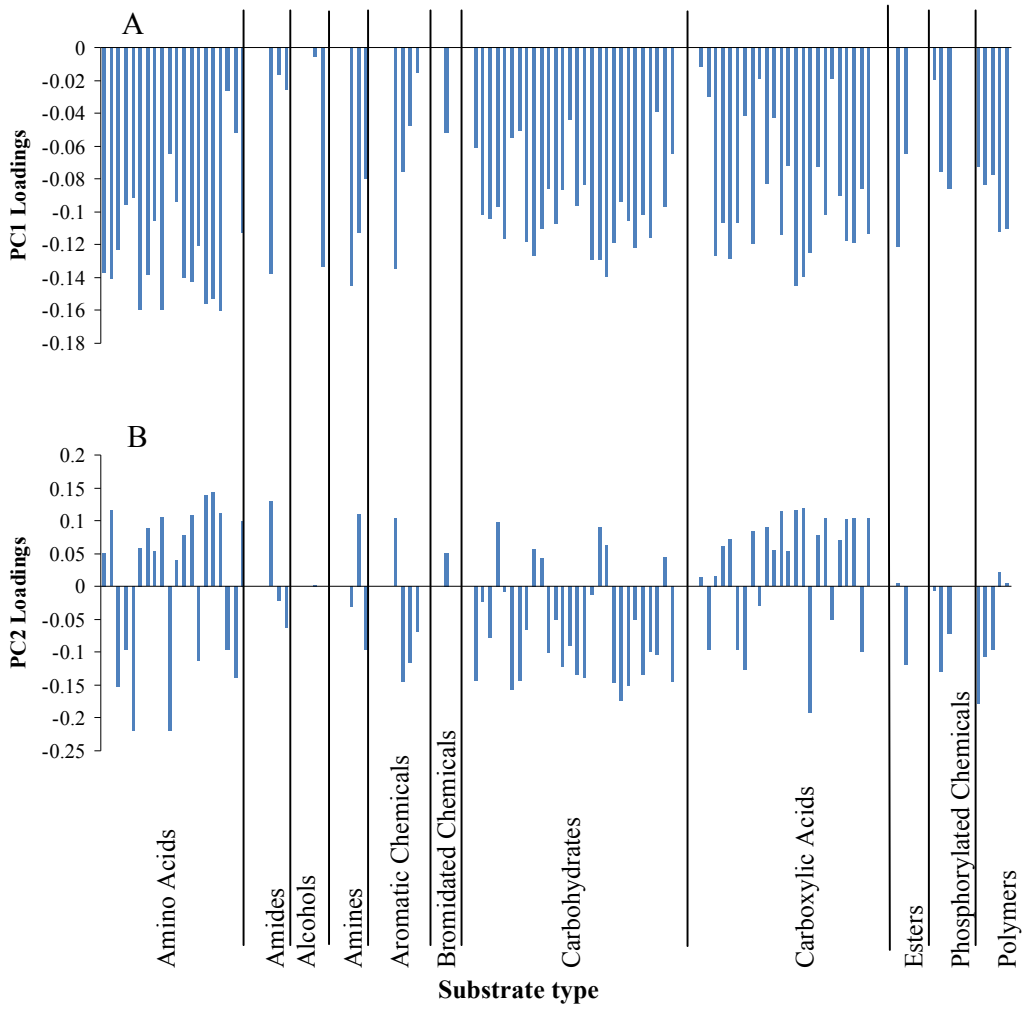


Figure 4.16: PC loadings for A) PC1 and B) PC2 of background-corrected AWCD (natural log transformed) data from the 95 carbon substrates in Biolog GN2 microtitre plates incubated over 120 hours.

PC scores for the samples were analysed by ANOVA, with soil dilution level, treatment type and harvest period as factors (Table 4.5). Biolog substrate utilisation patterns were significantly influenced by treatment type (i.e. bare soil, planted with and without AMF), harvest period and incubation time of the Biolog microtitre plate (Figure 4.17). Bare soil treatment had a higher mean PC1 score (due to the mainly positive PC1 scores) compared to the planted macrocosms with and without AMF (treatment effect, $F_{2,57} = 67.36$, $P < 0.001$; Figure 4.17A). More negative PC1 scores account for greater colour development; therefore bacteria isolated from the planted AMF and non-AMF treatments produced less colour in the Biolog plates than bare soil communities. PC2 scores were significantly influenced by treatment with the planted non-AMF treatment having the highest PC2 score with the bare soil treatment having a mean of almost zero compared to the planted treatments with AMF which had a negative mean PC2 score ($F_{2,57} = 58.18$, $P < 0.001$). The planted non-AMF soil had positive PC2 scores, that accounted for preferential utilisation of carboxylic acids and amides (which had positive PC2 loadings) whereas the bare soil treatment which had PC2 scores close to zero; showed no bias towards any particular C source. The planted mycorrhizal treatment had negative PC2 scores suggesting preferential utilisation of aromatics, carbohydrates, esters, phosphorylated chemicals and polymers.

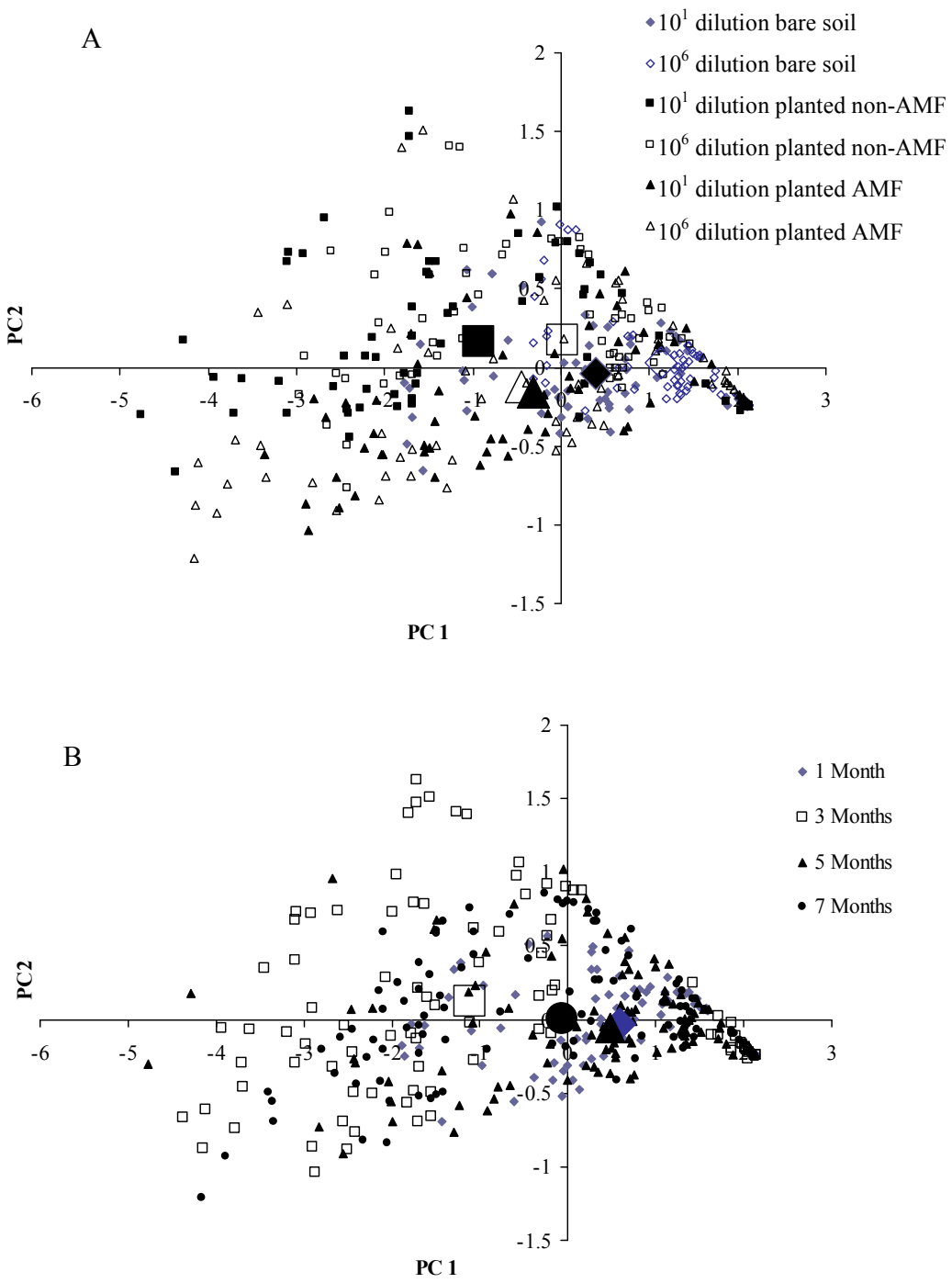


Figure 4.17: Ordination plot of PC scores produced from principal component analysis of transformed AWCD data from Biolog plates incubated for 120 hours for A) each treatment and B) each harvest period.
Large symbols represent mean PC score centroids.

As mentioned above, harvest time had a significant impact on PC1 and PC2 scores (Table 4.5). Mean PC1 scores were highest in the first and fifth month harvest (i.e. mainly positive (Figure 4.18)), compared to the third month harvest that had a predominantly negative PC1 scores. This suggests that colour development was greater in the third month harvest compared to the first and fifth month. Mean negative PC2 scores for the first and fifth month harvests suggest a bias towards utilisation of aromatics, carbohydrates, esters, phosphorylated chemicals and polymers. In comparison the third month harvest had the highest PC2 score reflecting the preferential use of amides and carboxylic acids. The final seventh month harvest had PC2 scores close to zero suggesting no bias towards any substrate guild.

Incubation time of the Biolog microtitre plates also significantly influenced PC1 and PC2 scores (Table 4.5; Figure 4.18). As expected the mean PC1 score became more negative with incubation time, suggesting that colour intensity across the microtitre wells increased with incubation time. The change in mean PC2 scores had a less obvious pattern than the PC1 scores, with PC2 scores being highest at 48 hours incubation followed by 72 hours (both time periods being predominantly positive) suggesting preferred utilisation of amides and carboxylic acids. Mean PC2 scores were lowest (and negative) at 24 hours followed by 96 and 120 hours incubation, suggesting a shift in substrate utilisation with time, with negative PC2 scores highlighting a bias of substrate utilisation towards aromatics, carbohydrates, esters, phosphorylated chemicals and polymers.

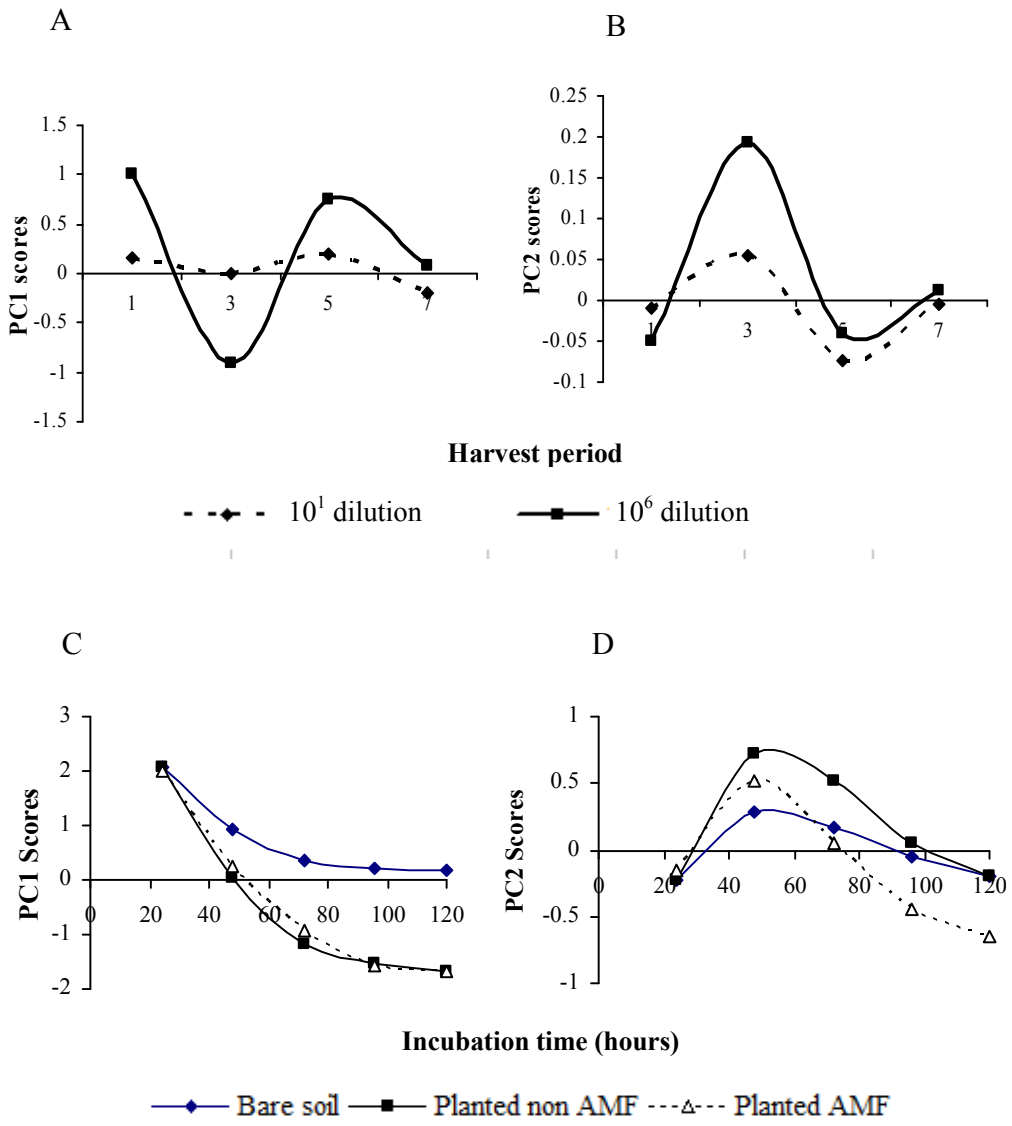


Figure 4.18: Interaction between soil dilution and harvest period on A) PC1 and B) PC2 score and the interaction between treatment type and incubation time on C) PC1 and D) PC2 scores.

Table 4.5: Significant results from an ANOVA of PC1 and PC2 scores against dilution level, treatment, harvest period and Biolog incubation time.

Source of variation	DF	F	P
PC1			
Dilution	1	28.61	< 0.001
Treatment	2	67.36	< 0.001
Harvest period	3	64.85	< 0.001
Dilution x Treatment	2	11.94	< 0.001
Treatment x Harvest period	6	5.02	< 0.001
Residual	*57*		
Incubation time	4	1107.42	< 0.001
Incubation time x Dilution	4	20.93	< 0.001
Incubation time x Treatment	8	43.63	< 0.001
Incubation time x Harvest	12	26.36	< 0.001
Incubation time x Dilution x Treatment	8	10.96	< 0.001
Incubation time x Treatment x Harvest period	24	5.14	< 0.001
Residual	*240*		
PC2			
Treatment	2	58.18	< 0.001
Harvest period	3	11.49	< 0.001
Residual	*57*		
Incubation time	4	789.53	< 0.001
Incubation time x Treatment	8	65.35	< 0.001
Incubation time x Harvest period	12	53.06	< 0.001
Incubation time x Dilution x Treatment	8	6.05	< 0.001
Incubation time x Dilution x Harvest period	12	2.25	0.030
Incubation time x Treatment x Harvest period	24	3.35	< 0.001
Incubation time x Dilution x Treatment x Harvest period	24	1.74	0.049
Residual	*240*		

Background soil dilution level had a significant effect on PC1 scores (Table 4.5), however no significant difference was observed for PC2 scores. Mean PC1 scores were lowest in the 10^1 dilution, having a predominantly negative score compared to the 10^6 dilution which was positive. This suggested that the highest colour intensity was seen in the microtitre plates at the 10^1 dilution, since PC1 was negative.

4.3.7 TERMINAL-RESTRICTION FRAGMENT LENGTH POLYMORPHISM

T-RFLP was carried out on DNA extracted from soil microbes under each dilution level and treatment at each harvest period. As mentioned in section 2.3.3.4 relative abundance of each peak occurring at a dye signal greater than 100 fluorescent units was assessed, with any shoulder peaks (associated with base pair addition through the use of PCR amplification) removed from analysis.

4.3.7.1 Species richness based on T-RFLP

The occurrence of peaks at certain fragment lengths determined from T-RFLP allowed determination of the species richness within each soil treatment at each harvest period. Fungal species richness within planted macrocosms with and without AMF had significantly more species with 14 recorded in both treatments compared to 10 species from the bare macrocosms (treatment as a single factor, $F_{2,47} = 5.03$, $P = 0.010$). Bare soil amended with the 10^1 dilution and the planted soils gave the most consistent results temporally after month one (dilution x treatment x harvest interaction, $F_{6,47} = 4.63$, $P < 0.001$; Figure 4.19).

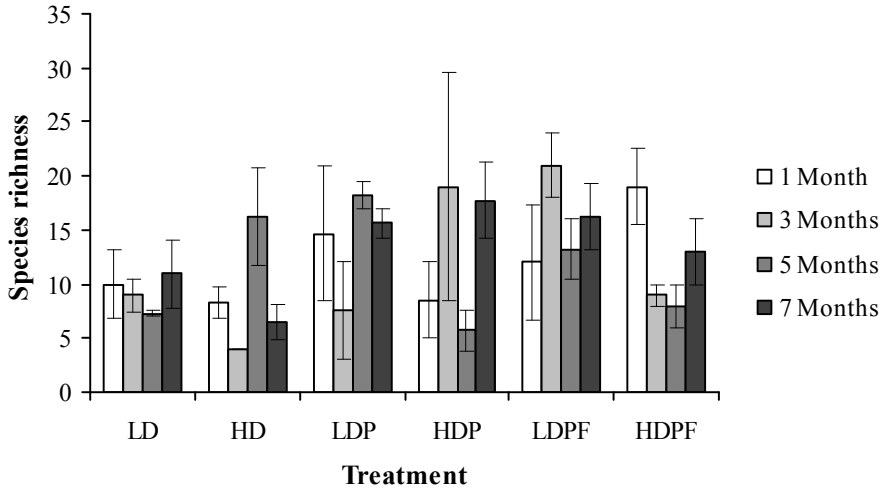


Figure 4.19: Fungal species richness for all macrocosms with each treatment and over each harvest period.

Species richness refers to the number of TRFs *Data are means ± standard errors.*

(Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

Bacterial species richness was significantly higher within the planted treatment without AMF (12 species) compared to that of the planted treatment + AMF which (9 species) and the bare soil treatments with 10 species ($F_{2,50} = 4.95$, $P = 0.011$). Species richness declined with time with 15 species recorded after one month but only 10 in months three, five and seven ($F_{3,50} = 15.62$, $P < 0.001$; Figure 4.20).

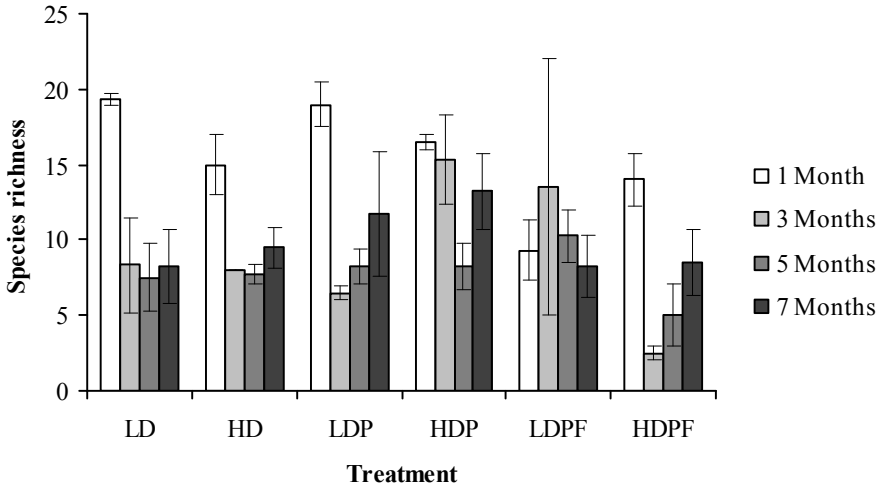


Figure 4.20: Bacterial species richness for all macrocosms with each treatment and over each harvest period.
Species richness refers to the number of TRFs.Significant dilution x treatment x harvest period interaction ($F_{6,50}$ = 3.72, P = 0.004) *Data are ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).*

4.3.7.2 Diversity index based on T-RFLP

Determination of Simpson's diversity index from T-RFLP relative abundance data provided an insight into the effect dilution level, treatment and harvest had on bacterial and fungal diversity. Fungal diversity was highest within the planted macrocosm containing AMF (0.84) compared to that of both the bare soil and planted non-AMF treatment (0.78 and 0.79 respectively) ($F_{2,47}$ = 3.49, P = 0.038). Simpson's diversity index was also influenced by harvest period with the highest diversity index of 0.85 found at the final seventh month harvest ($F_{3,47}$ = 3.94, P = 0.014). This was significantly higher than the third month harvest, when fungal diversity was at its lowest (0.75). Furthermore diversity within the 10^1 dilution planted soil was higher in the final seventh month harvest than in initial first month analysis, whereas the reverse

was true for the 10^6 dilution planted macrocosms, with the final month harvest having a high species richness than the first (dilution x treatment x harvest interaction, $F_{6,47}$ = 3.80, P = 0.004; Figure 4.21). In the planted macrocosms +AMF, species diversity was highest after three months within the 10^1 dilution amended soil, compared to the 10^6 dilution soil where species diversity was highest in the initial first harvest.

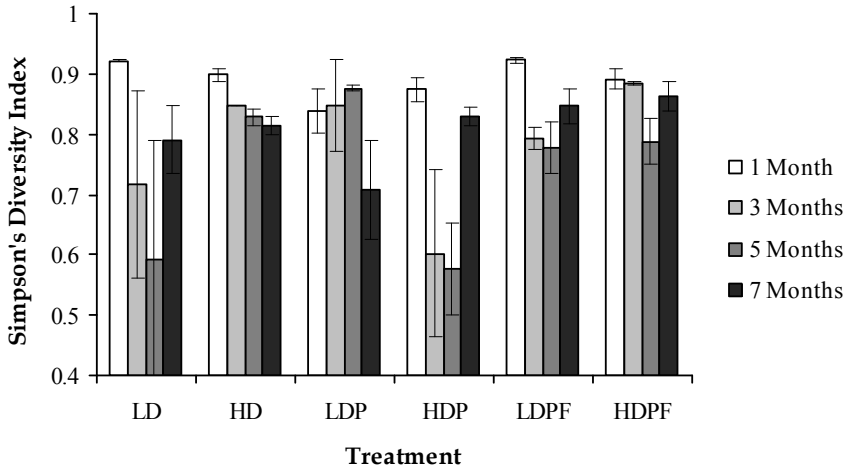


Figure 4.21: Fungal diversity index calculated using Simpson's equation for all treatments and at each harvest period.
Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

The highest level of bacterial diversity was observed within the first month harvest (0.89) compared to the later harvest periods ($F_{2,51}$ = 2.54, P = 0.002). Within the planted macrocosms +AMF, species diversity was highest within the 10^1 dilution compared to that of the 10^6 dilution (0.81 and 0.72 respectively) (dilution x treatment interaction, $F_{2,51}$ = 4.04, P = 0.023). This effect was reversed however within the bare soil treatment (with a lower species diversity in the 10^1 dilution compared to the 10^6 dilution treated macrocosms (0.75 and 0.84 respectively)). No effect of dilution level was observed within the planted non-AMF macrocosms. Closer examination shows at

month one, bacterial diversity is higher within the 10^1 dilution compared to the 10^6 within both bare soil and planted non-AMF treatment (dilution x treatment x harvest interaction, $F_{6,51} = 3.06$, $P = 0.012$; Figure 4.22).

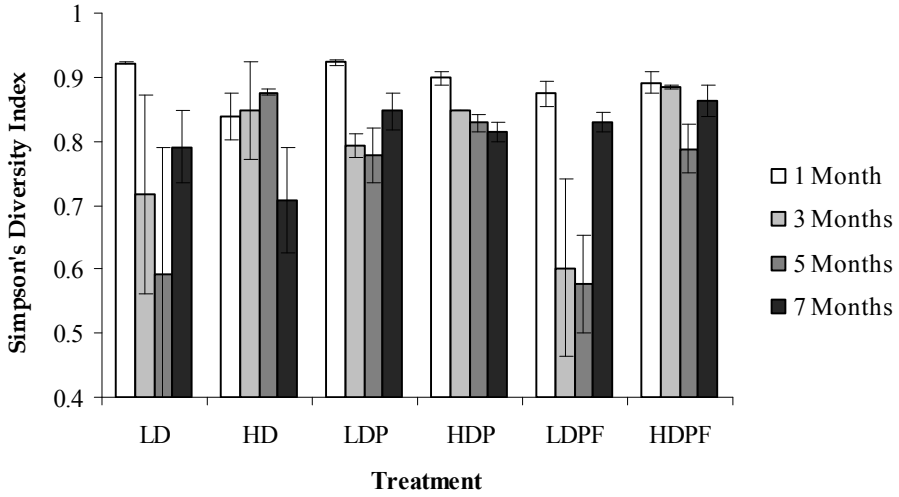


Figure 4.22: Bacterial diversity index calculated using Simpson's equation for all treatments and at each harvest period.
Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

4.3.7.3 Bacterial and fungal relative abundance

Through analysis of the relative abundance of common fragment lengths within the samples, it is clear to see that fungal communities were influenced by treatment with fragment 103 bp dominating the macrocosms that contained bare soil and the planted non-AMF macrocosms ($F_{2,47} = 14.57$, $P < 0.001$; Figure 4.23). Furthermore dilution level had a differing effect on the presence of fragment 75 bp with treatment, since the bare soil and planted +AMF had a highest occurrence at the 10^1 dilution, whereas within the planted non-AMF occurrence was greatest in the 10^6 dilution.

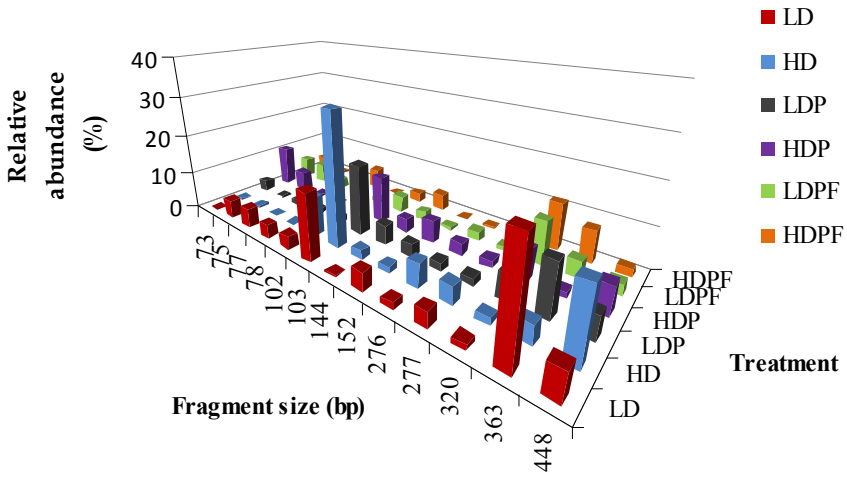


Figure 4.23: Relative abundance of the most commonly found fungal fragments within T-RFLP profiles.

No standard error bars are shown for clarity. *(Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).*

Bacterial fragments present were influenced across the differing dilution and treatments (Figure 4.24) with both fragments 340 and 369 bp highest in the 10^6 bare soil, whereas within the planted (±AMF) treatments their presence was highest within the 10^1 dilution ($F_{2,50}$ = 27.72, P < 0.001 and $F_{2,50}$ = 19.58, P < 0.001 respectively). Such a pattern was also observed for fragment 405 bp, which was more abundance in the 10^6 bare soil treatment, whereas in the planted +AMF treatment abundance was higher at the 10^1 dilution ($F_{2,50}$ = 16.83, P < 0.001). Further effects of treatment and dilution were observed through PCA analysis as described in the following section.

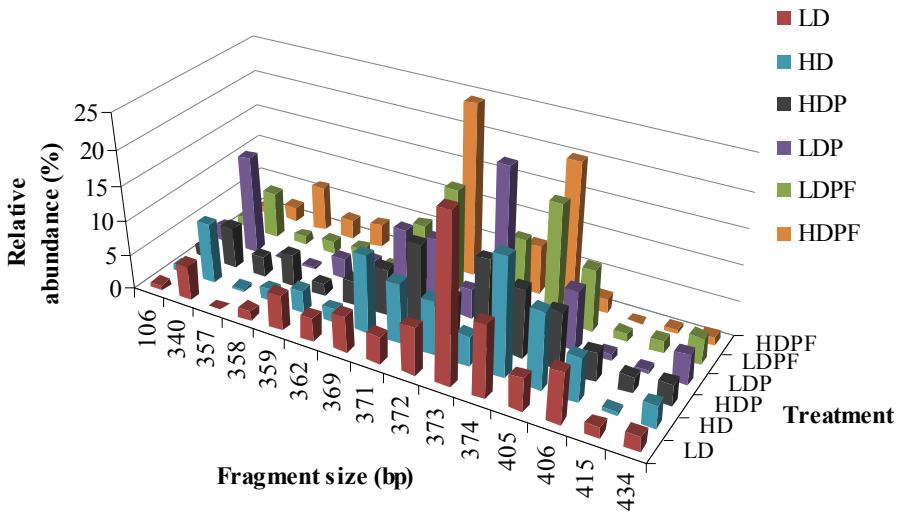


Figure 4.24: Relative abundance of the most commonly found bacterial fragments within T-RFLP profiles.

No standard error bars are shown for clarity. *(Treatment codes LD and HD = 10^{1} and 10^{6} in a bare soil treatment; LDP and HDP = 10^{1} and 10^{6} dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^{1} and 10^{6} dilution in a planted AMF treatment).*

4.3.7.4 PCA analysis of fungal T-RFLP

Principal component analysis using the covariance matrix was carried out on the relative abundance of all T-RFLP fragment profiles, but analysing bacteria and fungi separately. For the fungal data set principal component 1 (PC1) accounted for 29.2 % of the total variation within the macrocosms, PC2 accounted for 16.88 % and PC3 accounted for 12.35 % (Table 4.6). These first three principal components explained a total variation of 58.43 %.

Table 4.6: Results of principal component analysis of fungal T-RFLP relative abundance for each treatment and at each harvest period for each macrocosm.

	PC1	PC2	PC3
Eigenvalues	464.5	268.5	196.5
% Variance	29.2	16.88	12.35
Cumulative	29.2	46.08	58.43

Factor loadings, describe which fragments contribute the most variation in the principal component analysis. Factor loading values were analysed for all fragments to ascertain which were making a significant contribution to PC1, PC2 and PC3 (Figure 4.25). The fragments with the highest loading values in each PC axis were identified and any fragments with PC loading values > ±0.25 were classed as significant (Pio *et al*., 1996). PC1 can therefore be described as the presence and absence of one fragment, PC2 is determined by three fragments and PC3 by four fragments. For example PC1 shows that when a sample profile contains a fragment of 103 bp, it would therefore have a negative PC1 score whereas if the PC1 score was positive fragment 103 bp would not be found.

Figure 4.26 is an ordination plot of PC1 versus PC2 scores from each dilution amendment, treatment and harvest. An ANOVA of PC1 scores showed that treatment and harvest period had a significant effect on PC1 (Table 4.7). Microbial DNA profiles from the planted AMF treatment had a positive PC1 score (8.52), which suggests the absence of fragment 103 bp (this was also true for the planted non-AMF macrocosms but not to the same degree, since the PC1 score was less positive (1.18)). The bare soil treatment had a negative PC score (-8.67) reflecting the dominance of fragment 103 bp. As mentioned above, harvest period also had a significant effect on PC1 scores, with the first two harvest periods having negative scores (-14.07 for the first and -1.32 for the third) compared to that of the fifth and seventh month (1.32 and 10.34 respectively) suggesting the presence of the 103 bp fragment at the start of the experiment (Figure 4.27). Additional interactions within the data set can be seen in Table 4.7.

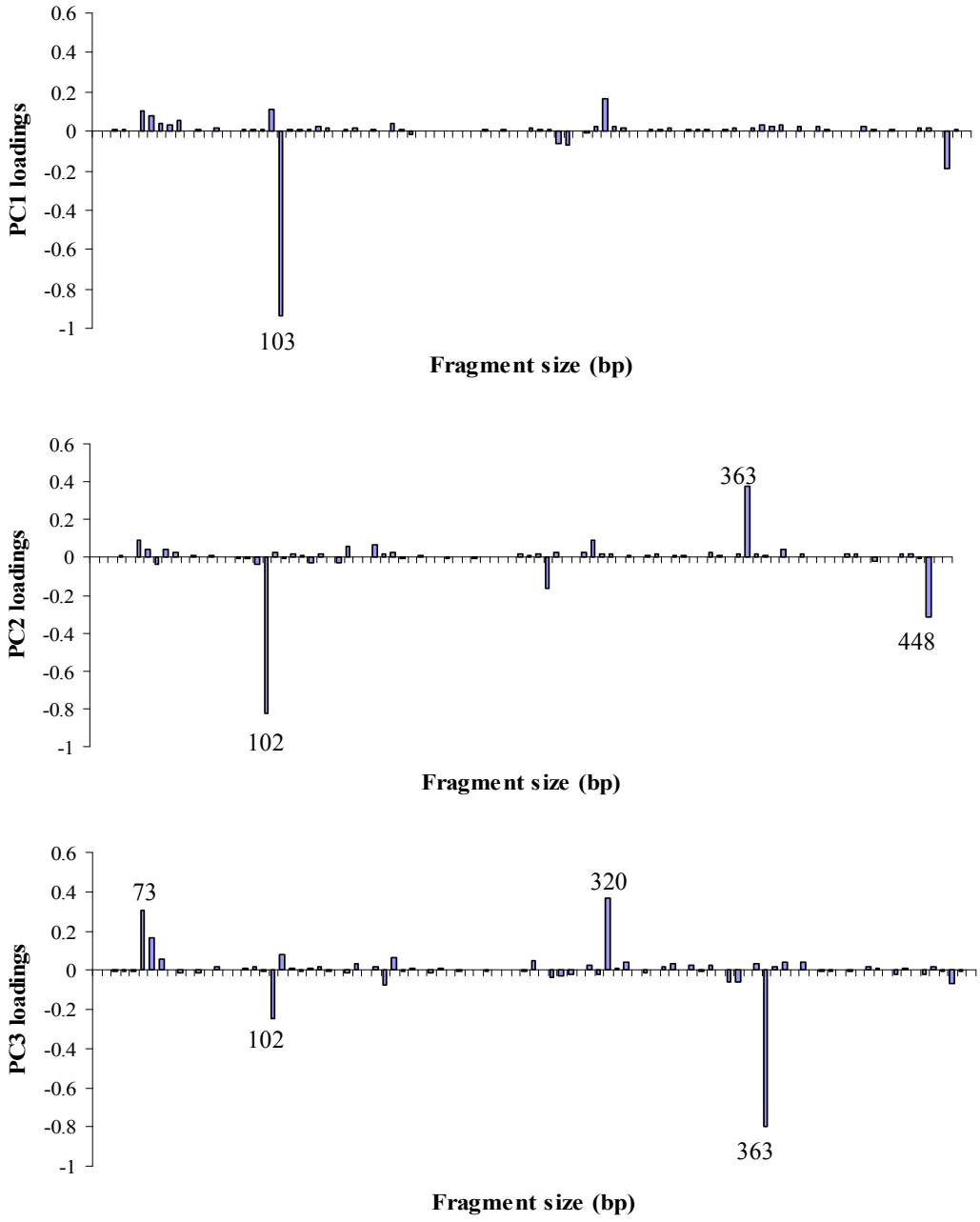


Figure 4.25: PC loadings for the first three principal component (PC) from relative abundance data collected from T-RFLP analysis of fungal communities.

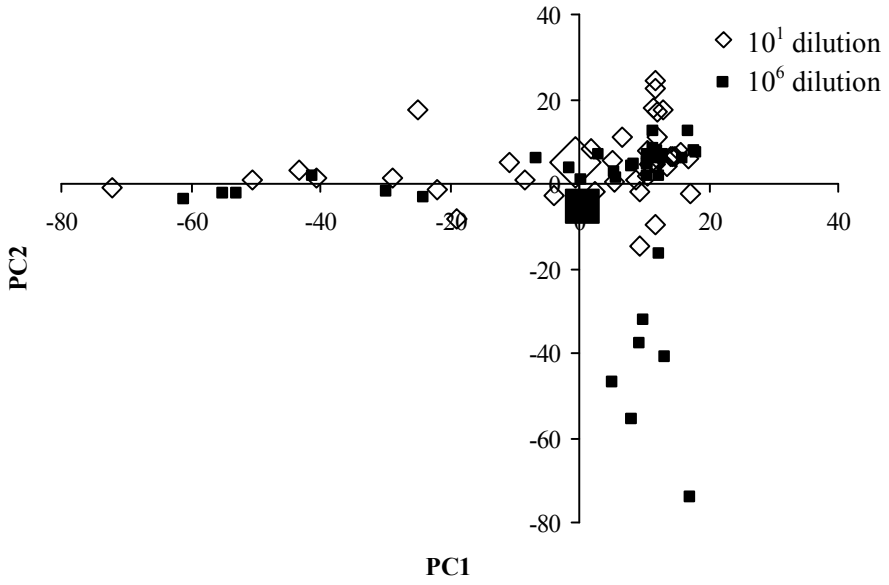


Figure 4.26: Ordination plot of PC1 versus PC2 scores for fungal fragments of various sizes from each treatment at each harvest.
Large symbols represent mean PC score centroids.

PC2 scores for each sample clustered with dilution level (Table 4.7). PC2 shows that when a sample profile contains fragment of 363 bp, it would therefore have a positive PC2 score whereas if the PC2 score was negative fragment lengths 102 and 448 bp would be found instead of 363 bp. Hence, in terms of dilution level, within the 10^1 dilution level (whose PC2 score was 4.85) fragment 363 bp would be found instead of 102 and 448 bp, whereas the reverse would be true within the 10^6 dilution macrocosms, whose PC2 score was -5.41. Additional interactions within the PC2 dataset can be seen in Table 4.7.

Within the 10^1 dilution soil, PC3 scores were negative (-2.69) compared to those of the 10^6 dilution added soil (2.96) (Table 4.7). With respect to the effect of treatment, the PC3 score was negative within the bare soil treatment (-8.69) compared to that of the

planted AMF and non-AMF treatments which had positive PC3 scores (5.63 and 3.86 respectively; Table 4.7; Figure 4.27). Finally within the first and seventh month harvest negative PC3 scores (-0.81 and -4.19 respectively) compared to those of the third and fifth month harvest where PC3 scores were 0.16 and 4.72 respectively (Table 4.7; Figure 4.27). Assessment of the PC3 loadings given in Figure 4.25, therefore implies the occurrence of a negative PC3 score reflects the presence of fragment lengths 73 and 320 bp and the absence of 102 and 363 bp, whereas a positive PC3 score reflects the reverse pattern.

Additional interactions that had a significant impact on PC1, PC2 and PC3 scores was the interaction of dilution x treatment x time which can be seen in Figure 4.27 with other interactions observed in Table 4.7.

Within the 10^1 dilution soil, PC3 scores were negative compared to those of the 10^6 dilution amended soil (-1.50 and 2.03 respectively; Table 4.9). With respect to the effect of treatment, PC3 scores were negative within the bare soil treatment (-4.95) compared to those of the planted AMF and non-AMF treatments which had positive PC3 scores (1.89 and 3.78 respectively) (ANOVA of PC3 scores; Table 4.9; Figure 4.30). Finally, within the first and third month harvest, PC3 scores were positive (9.18 and 6.66 respectively) compared to those of the fifth and seventh month harvest where PC3 scores were -3.75 and -5.87 respectively (Table 4.9; Figure 4.30). Assessment of the PC3 loadings given in Figure 4.30, implies the occurrence of a negative PC3 score reflects the presence of fragment lengths 373, 374 and 406 bp with the absence of 371

bp, whereas a positive PC3 score reflects the reverse of this. Additional interactions within the PC3 dataset can be seen in Table 4.9. Table 4.10 show presumptive identification of T-RFLP fragments making significant contributions to the three PC axes. The similar size of fragments 371, 373, and 374 bp suggests a commonality of family or genus rather than distinct species.

Table 4.10 show presumptive identification of T-RFLP fragments making significant contributions to the three PC axes. The similar size of fragments 102 and 103 bp suggests a commonality of family or genus rather than distinct species.

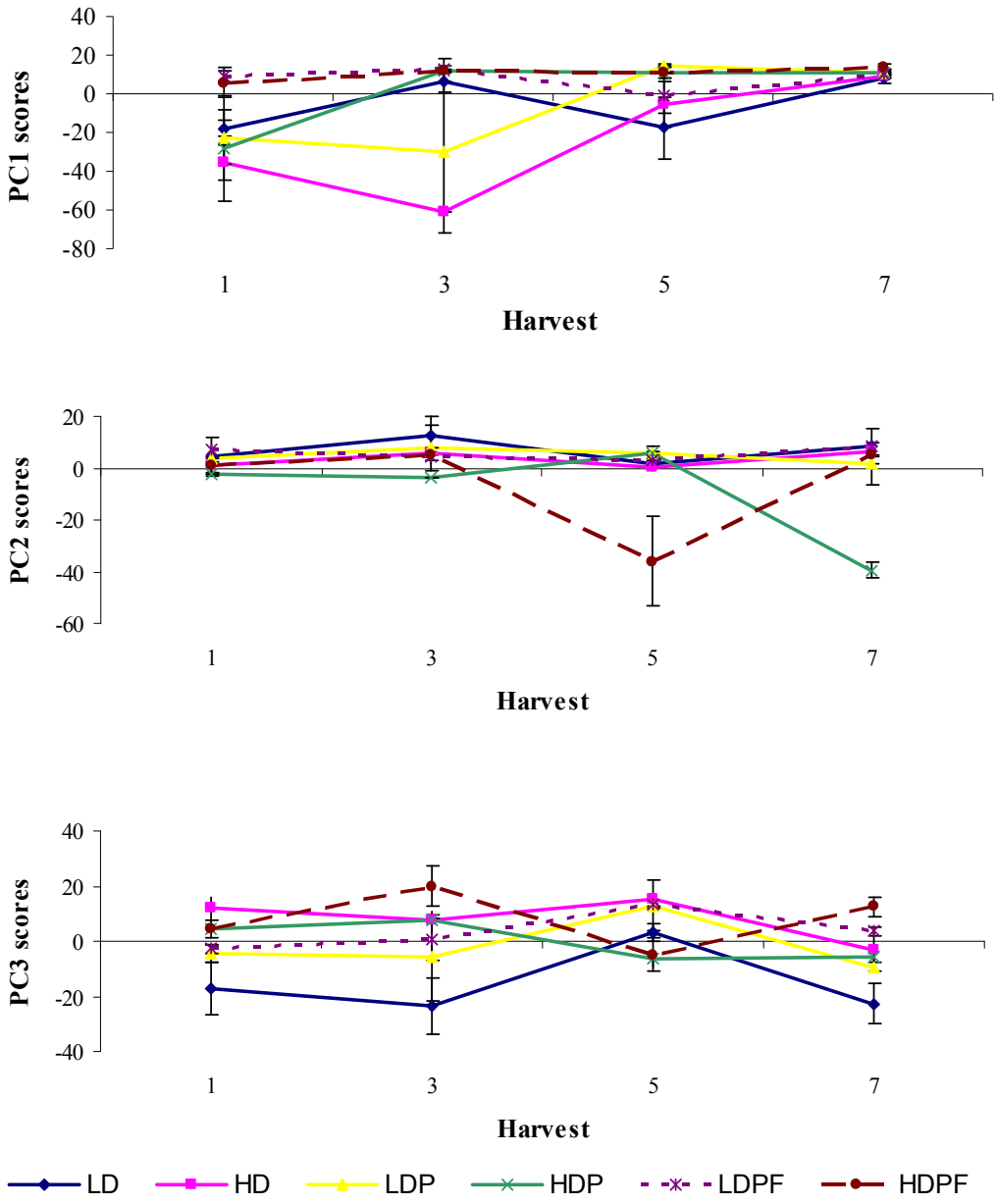


Figure 4.27: Effect of treatment and harvest on PC1, PC2 and PC3 scores for fungal T-RFLP dataset.

Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

Table 4.7: Results from ANOVA for PC1, PC2 and PC3 scores originating from fungal data from T-RFLP profiles.

Source of variation	DF	F	P
PC1			
Treatment	2	16.47	< 0.001
Harvest	3	11.28	< 0.001
Dilution x Treatment	2	4.88	0.012
Treatment x Harvest	6	3.69	0.004
Dilution x Treatment x Harvest	6	5.79	< 0.001
Residual	*47*		
PC2			
Dilution	2	14.33	< 0.001
Treatment	3	3.00	0.060
Harvest	3	2.71	0.056
Dilution x Treatment	2	6.33	0.003
Treatment x Harvest	6	4.73	< 0.001
Dilution x Treatment x Harvest	6	6.87	< 0.001
Residual	*47*		
PC3			
Dilution	1	11.83	0.001
Treatment	2	17.62	< 0.001
Harvest	3	4.17	0.011
Dilution x Treatment	2	8.29	< 0.001
Dilution x Harvest	3	8.91	< 0.001
Diversity x Treatment x Harvest	6	3.69	0.004
Residual	*47*		

4.3.7.5 PCA analysis of bacterial T-RFLP

For the bacterial data set principal component 1 (PC1) accounted for 34.63 % of the total variation within the macrocosms, with PC2 accounting for 15.93 % and PC3 11.03 % (Table 4.8). These first three principal components explained a total variation of 61.59 %.

Table 4.8: Results of principal component analysis of bacterial T-RFLP relative abundance for each treatment and at each harvest period for each macrocosm.

	PC1	PC2	PC3
Eigenvalues	454.4	209.1	144.7
% Variance	34.63	15.93	11.03
Cumulative	34.63	50.56	61.59

As previously, factor loading values were analysed for all fragments (with any T-RFs with PC loading values > ±0.25 deemed as significant (Pio *et al*., 1996)) to ascertain which were making a noteworthy contribution to PC1, PC2 and PC3 (Figure 4.28). PC1 can therefore be described as the presence and absence of two fragments, PC2 is determined by four fragments and PC3 by four fragments. For example PC1 shows that when a sample profile contains fragment of 374 bp, it would therefore have a positive PC1 score whereas if the PC1 score was negative fragment 373 bp would be found instead of 374 bp.

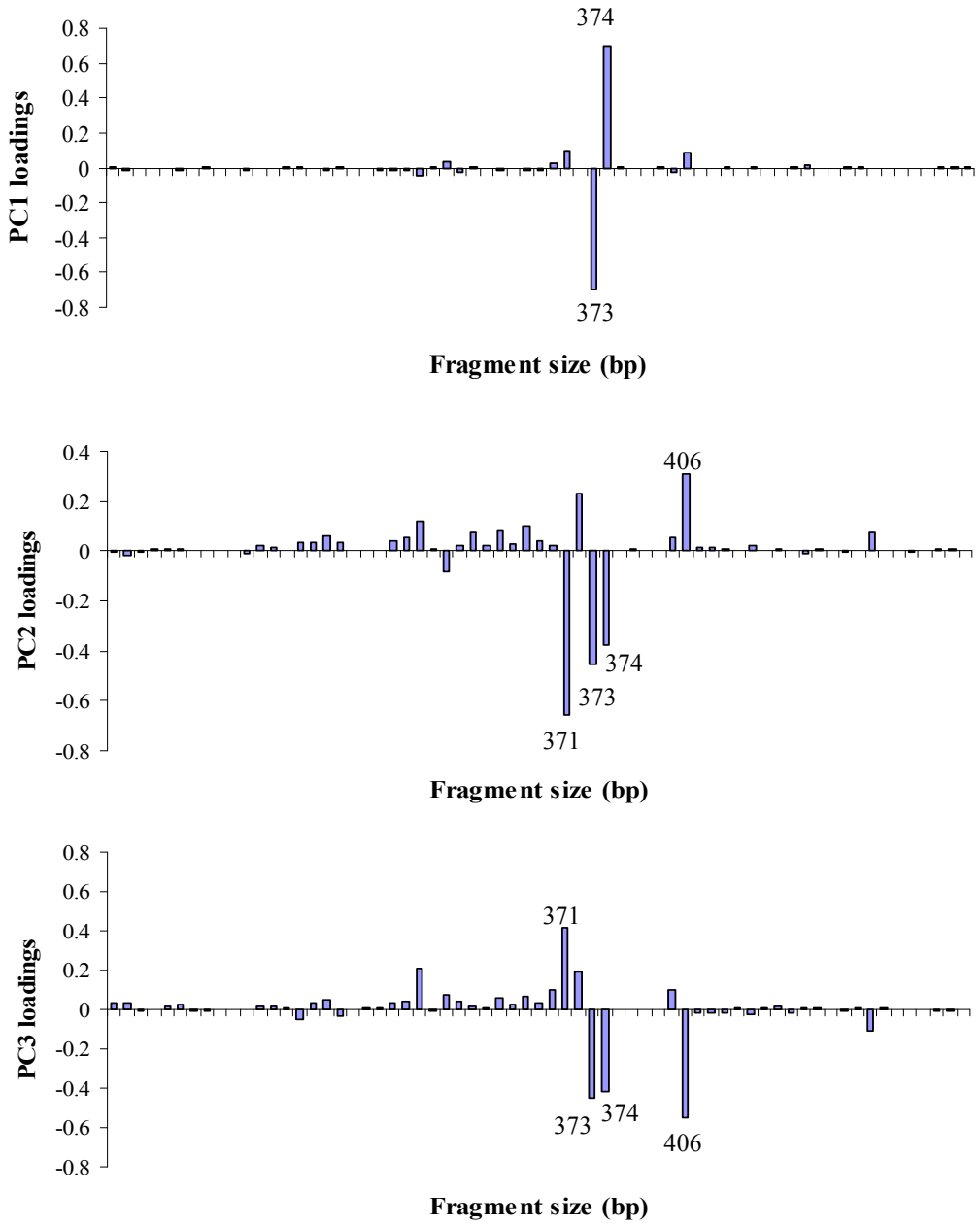


Figure 4.28: PC loadings for the first three principal components (PC) from relative abundance data collected from T-RFLP analysis of bacterial communities.

Figure 4.29 is an ordination plot of PC1 versus PC2 scores from each dilution amendment, treatment and harvest. PC1 scores were significantly influenced by

dilution, treatment and harvest period (ANOVA of PC1 scores; Table 4.9; Figure 4.30). Microbial DNA profiles from the 10^6 dilution macrocosms had a positive PC1 score (5.50) which suggests the presence of fragment 374 bp and the absence of 373 bp, whereas in the 10^1 dilution macrocosms the reverse would be true (with a PC1 score of -5.45). Such patterns can be clearly observed in an ordination plot shown in Figure 4.29. As mentioned above, treatment also influenced PC1 scores, with planted AMF treatments having a positive PC1 score (8.60), reflecting the presence of fragment 374 bp and the absence of fragment 373 bp. Whereas, the planted non-AMF treatment whose PC score was negative (-8.67) reflected the dominance of fragment 373 bp and absence of 374 bp (this was also true for the bare soil macrocosms but not to the same degree, since the PC1 score was less negative (-0.37)). Furthermore, harvest period had a significant effect on PC1 scores, with the first, third and fifth harvest periods all having negative scores (-4.66, -4.32 and -6.32 respectively) compared to that of the seventh month (11.09) suggesting the presence of the 373 fragment, until the final harvest when 373 bp becomes absent and 374 bp dominates. Additional interactions within the PC2 dataset can be seen in Table 4.9.

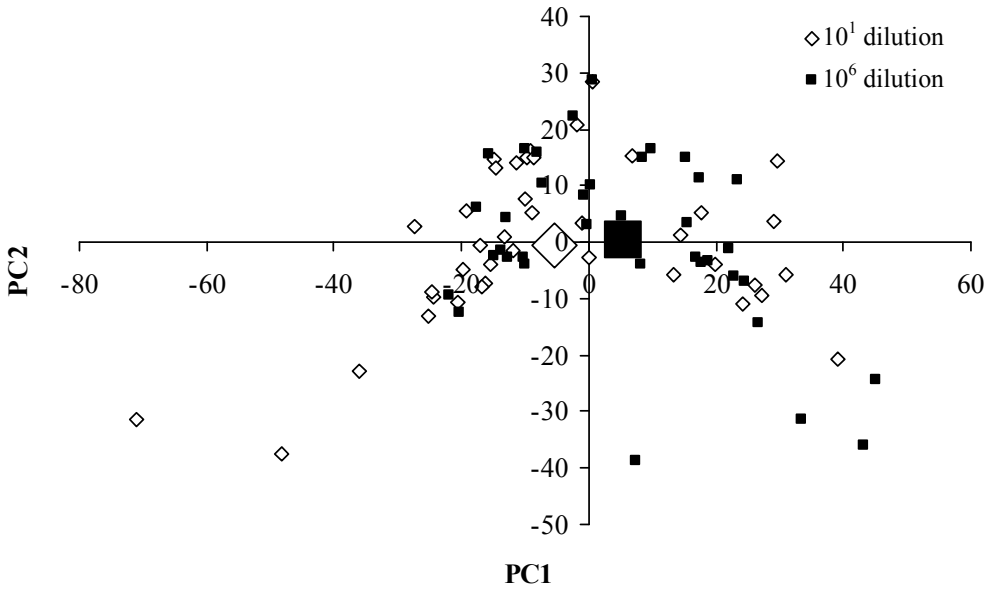


Figure 4.29: Ordination plot of PC1 versus PC2 scores for bacterial fragments of various sizes from each treatment at each harvest time.

PC2 scores clustered with treatment and harvest period (Table 4.9; Figure 4.30). PC2 showed that when a sample profile contains fragment length 406 bp, it would have a positive PC2 score whereas if the PC2 score was negative fragment lengths 371, 373 and 374 bp would be found instead of 406 bp. Hence, in terms of treatment, within the bare soil and also the planted non-AMF macrocosms (which have PC2 scores of 4.78 and 0.745) fragment 406 bp would be found instead of 371, 373 and 374 bp, whereas the reverse would be true within the planted AMF macrocosms, with a PC2 score of -6.31. In terms of harvest period PC2 score was only positive (10.86) in the first harvest suggesting fragment 406 bp would be found instead of 371, 373 and 374 bp, whereas the reverse would be true within the third, fifth and seventh harvest (whose PC2 scores were -7.12, -2.73 and -1.80 respectively). Additional interactions within the PC2 dataset can be seen in Table 4.9.

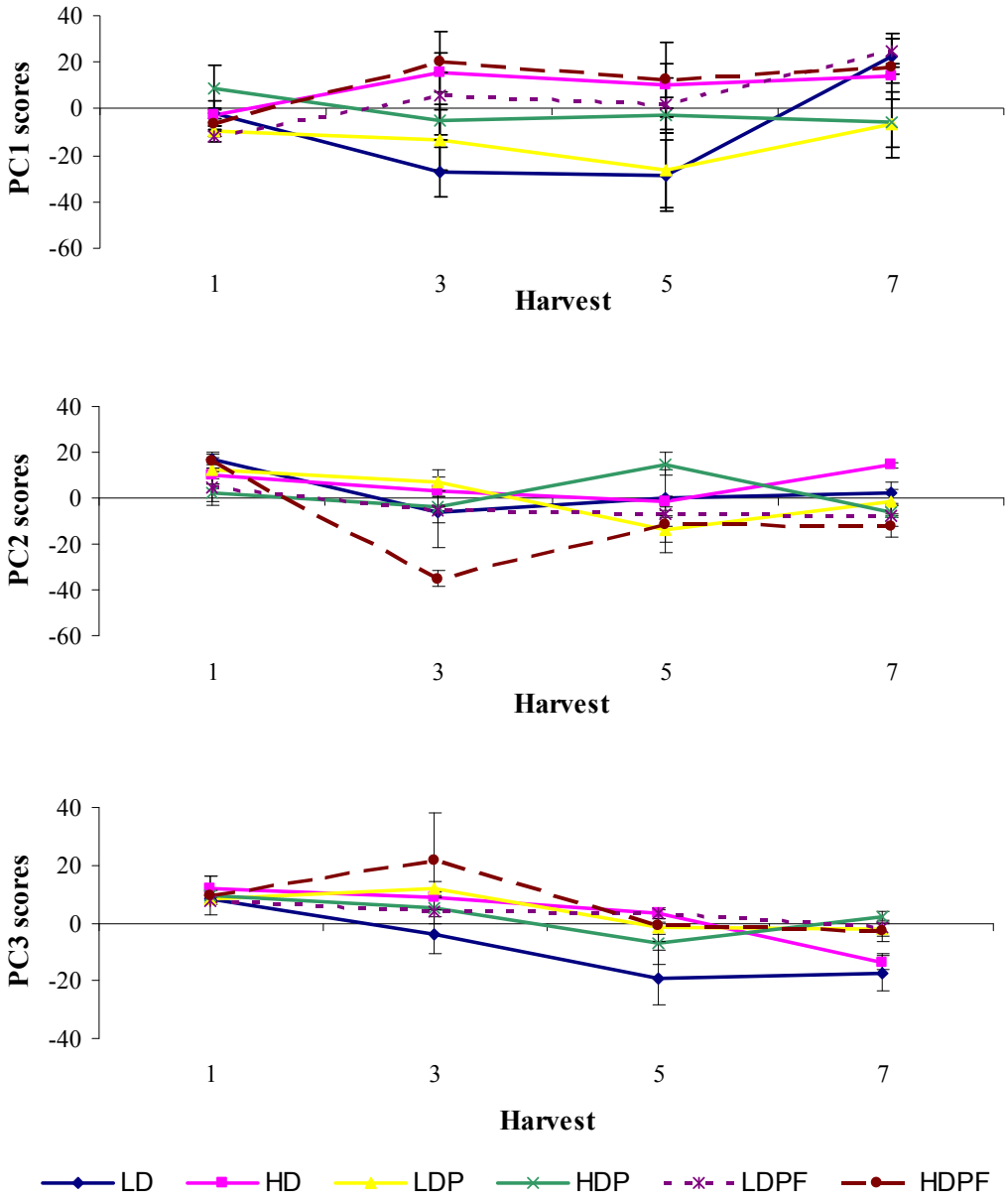


Figure 4.30: Effect of treatment and harvest on PC1, PC2 and PC3 scores for bacterial T-RFLP dataset.

Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

Table 4.9: Results from ANOVA of PC1, PC2 and PC3 of bacterial data from T-RFLP profiles.

Source of variation	DF	F	P
PC1			
Dilution	1	12.07	0.001
Treatment	2	6.43	0.003
Harvest	3	4.85	0.005
Dilution x Harvest	3	3.64	0.019
Treatment x Harvest	6	2.47	0.036
Residual	*50*		
PC2			
Treatment	2	9.58	< 0.001
Harvest	3	9.45	< 0.001
Dilution x Harvest	3	2.88	0.045
Treatment x Harvest	6	2.40	0.041
Dilution x Treatment x Harvest	6	4.66	< 0.001
Residual	*50*		
PC3			
Dilution	1	5.33	0.025
Treatment	2	7.03	0.002
Harvest	3	14.70	< 0.001
Dilution x Treatment	2	3.51	0.037
Diversity x Treatment x Harvest	6	2.45	0.037
Residual	*50*		

Within the 10^1 dilution soil, PC3 scores were negative compared to those of the 10^6 dilution amended soil (-1.50 and 2.03 respectively; Table 4.9). With respect to the effect of treatment, PC3 scores were negative within the bare soil treatment (-4.95) compared to those of the planted AMF and non-AMF treatments which had positive PC3 scores (1.89 and 3.78 respectively) (ANOVA of PC3 scores; Table 4.9; Figure 4.30). Finally, within the first and third month harvest, PC3 scores were positive (9.18 and 6.66 respectively) compared to those of the fifth and seventh month harvest where PC3 scores were -3.75 and -5.87 respectively (Table 4.9; Figure 4.30). Assessment of

the PC3 loadings given in Figure 4.30, implies the occurrence of a negative PC3 score reflects the presence of fragment lengths 373, 374 and 406 bp with the absence of 371 bp, whereas a positive PC3 score reflects the reverse of this. Additional interactions within the PC3 dataset can be seen in Table 4.9. Table 4.10 show presumptive identification of T-RFLP fragments making significant contributions to the three PC axes. The similar size of fragments 371, 373, and 374 bp suggests a commonality of family or genus rather than distinct species.

Table 4.10: Presumptive identification of T-RFLP fragments for fungi and bacteria that made significant contributions to the first three PC values of soil microbial communities from macrocosms.

Note: Not all fragments have presumptive identification as the current databases is incomplete and hence are listed as unknown.
Identification from database (Dickinson, pers. comm., 2009).

Fragment	Species	Genus	Order	Class
Fungal (ITS)				
73	*Fusarium culmorum*	*Fusarium*	Hypocreales	Sordariomycetes
102	Unknown A	-	-	-
103	*Rhizoctonia solani*	*Thanatephorus*	Cantharellales	Agaricomycetes
320	Unknown B	-	-	-
363	Unknown C	-	-	-
448	Unknown D	-	-	-
Bacterial (23S)				
371	?	*Burkholderia*	Burkholderiales	Betaproteobacteria
373	Unknown E	-	-	-
374	Unknown F	-	-	-
406	*Clostridium novyi*	*Clostridium*	Clostridiales	Clostridia

4.3.8 AGGREGATE SIZE DISTRIBUTION

Few differences in the aggregate size distribution (ASD) were observed over harvest period in Figure 4.31A-D. ASD at the first harvest was very similar regardless of

treatment and dilution. Differences between the treatments were observed at the third month, where the planted non-AMF treatment had a greater number of larger aggregates than the planted with AMF and bare soil treatments. The treatment containing the smallest number of aggregates at this time period was the 10^1 dilution amended bare soil macrocosm. In comparison at the fifth month harvest the planted AMF treatment at the 10^6 dilution had the largest quantity of aggregates present, whereas the 10^1 planted AMF treatment contained the lowest. At the final harvest, greater numbers of larger aggregates were present in the 10^6 planted non-AMF treatment, however this was closely followed by the 10^1 dilution. Dilution level appeared to have an effect on the ASD of the bare soil and planted AMF treatments, with fewer larger aggregates measured in the 10^6 dilution compared to the 10^1.

The bare soil treatment had a significantly higher ASD_{CU} (section 2.2.3) than the planted AMF treatment regardless of dilution level (treatment effect, $F_{2,57} = 3.96$, $P = 0.025$). In comparison, no difference was found between the bare soil and planted AMF macrocosms. In addition to treatment, harvest also had an influence on ASD_{CU}, with the first month harvest having a higher value than the third. An increase in ASD_{CU} took place between the fifth and third harvest, with no differences observed from the fifth to the final month.

Within the bare soil treatment the ASD_{CU} was higher at the 10^6 dilution compared to the 10^1 (dilution x treatment, $F_{2,57} = 5.33$, $P = 0.008$; Figure 4.32). Despite the ASD_{CU} being lower within the 10^6 than 10^1 dilution, no significant dilution x treatment

interaction was evident for planted AMF and non-AMF macrocosms. It is worth noting however that the ASD_{CU} was highest in the 10^6 bare soil treatment, followed by the planted non-AMF fungi at the 10^1 dilution and with the lowest ASD_{CU} found within the planted AMF macrocosm at the 10^6 dilution. At months five and seven the ASD_{CU} in the bare soil treatment was higher than that of the planted treatments (treatment x harvest interaction, $F_{6,57} = 2.85$, $P = 0.017$).

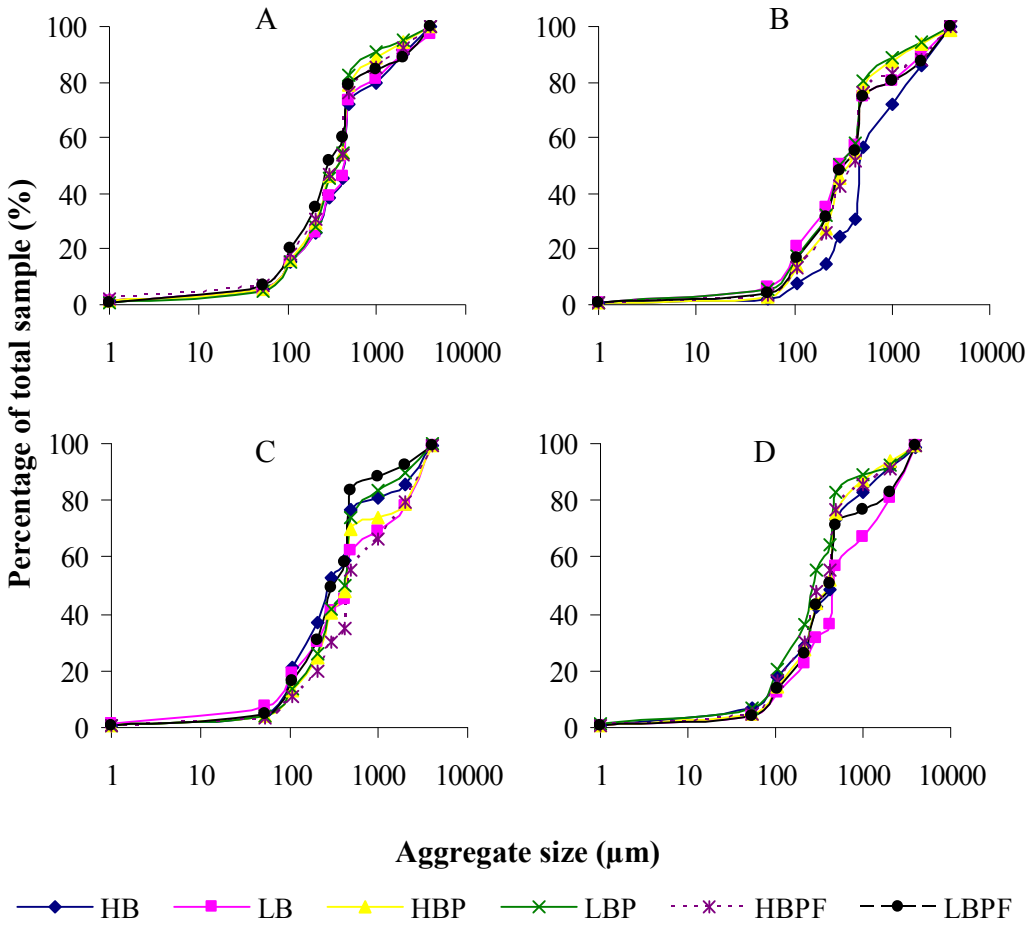


Figure 4.31: Aggregate size distribution for all treatments and dilutions at the A) first; B) third; C) fifth and D) seventh month harvest.

Standard errors are not shown for clarity.(Treatment codes HB and LB = 10^1 and 10^6 in a bare soil treatment; HBP and LBP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, HBPF and LBPF = 10^1 and 10^6 dilution in a planted AMF treatment).

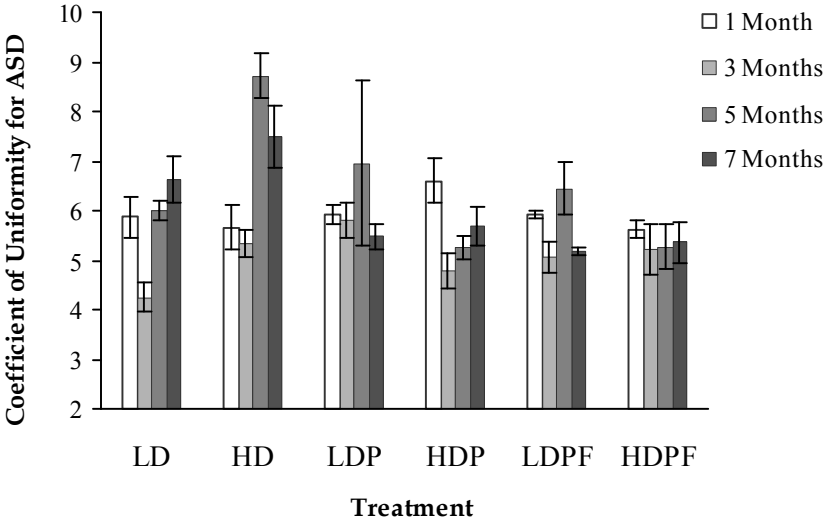


Figure 4.32: Coefficient of uniformity value for aggregate size distribution of the soil within the macrocosms at four harvest periods after inoculation and planting.
Dilution x treatment x harvest effect ($F_{6,57} = 2.68$, $P = 0.023$). *Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).*

4.3.9 AGGREGATE STABILITY

Macrocosms treated with the 10^6 dilution had a mean weight diameter (MWD) of 1.35 mm compared to that of 1.24 mm for soil amended with the 10^1 dilution (dilution effect, $F_{1,56} = 12.16$, $P < 0.001$). Aggregate stability significantly increased from the first to the third month, where the mean MWD was at its highest (1.90 mm). This value is just below the classification of a very stable aggregate (harvest effect, $F_{3,56} = 459.24$, $P < 0.001$). Thereafter, aggregate stability significantly declined at five months to the lowest MWD value of 0.89 mm after which a slight increase was observed. On inspection of the individual aggregates, there appeared to be little physical difference between the aggregates, apart from some slight surface morphological differences

(Figure 4.34). Such differences would not have been sufficient to cause such large changes in aggregate stability over time.

Aggregate stability was greatest in macrocosms containing plants +AMF relative to the planted -AMF and bare soil treatments (treatment effect, $F_{2,56}$ = 4.58, P = 0.014), particularly in soils amended with the 10^1 dilution (dilution x treatment interaction, $F_{2,56}$ = 4.82, P = 0.012). Planted macrocosms (-AMF) and bare soil treatments exhibited greater aggregate stability when amended with the 10^6 dilution (Table 4.11). There was a general trend across all treatments with a reduction in aggregate stability after three months although the plant mediated enhancement between one and three months gives rise to a treatment x harvest interaction ($F_{6,56}$ = 3.76, P = 0.003; Figure 4.34).

Figure 4.33: Example of aggregates taken from the 10^1 dilution, planted but non mycorrhizal macrocosm at one, three, five and seven months harvest.

Table 4.11: Mean aggregate stability under the two different dilution levels within the bare soil and planted macrocosms with and without AMF.

Data are means of replicates ±standard error

	Treatment		
Dilution	**Bare soil (mm)**	**Planted without AMF (mm)**	**Planted with AMF (mm)**
10^1 (Low)	1.19 ± 0.12	1.20 ± 0.13	1.34 ± 0.14
10^6 (High)	1.36 ± 0.10	1.31 ± 0.12	1.36 ± 0.14

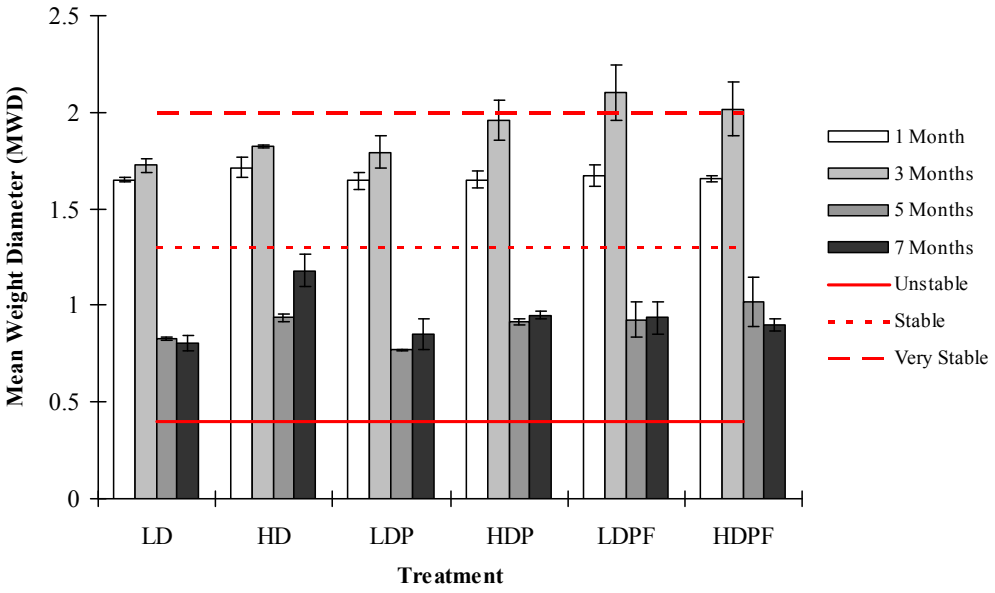


Figure 4.34: Aggregate stability of the soil within the macrocosms measured by MWD at four harvest periods after inoculation and planting.

Treatment x harvest effect ($F_{6,56}$ = 3.76, P = 0.003). *Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).*

4.3.10 AGGREGATE WATER REPELLENCY

The first and third harvest had similar repellency values (2.01 and 1.96 respectively). Aggregates taken from the fifth month harvest had the highest R index value (2.39)

although this was reduced by month seven (2.14) (harvest as a single factor, $F_{3,55}$ = 5.60, P = 0.002; Figure 4.35).

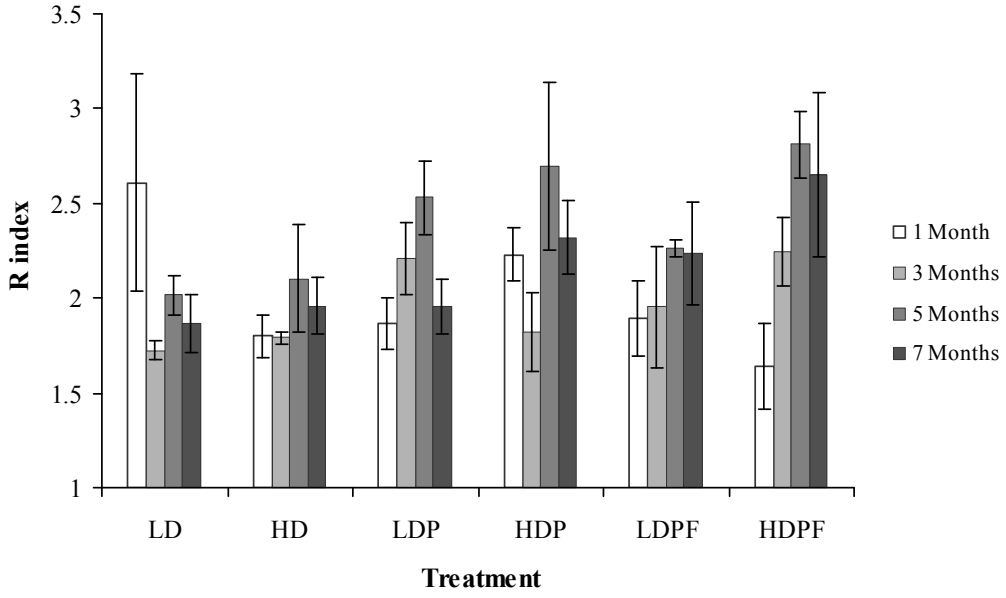


Figure 4.35: Aggregate water repellency of the soil within the macrocosms measured by the R index at four harvest periods after inoculation and planting.

Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

4.3.11 MESOSCALE VISUAL EVALUATION OF SOIL STRUCTURE

Figures 4.36-4.39 show images used for pore size and morphological determination. Visual assessment of these images allows the changes of pore space (such as size and distribution) within the soil macrocosms to be observed. Images obtained from the bare soil treatment at the third month harvest show the effect soil settling had on pore spaces within the bulk soil over time (particularly within the 10^1 dilution) which contained fewer pores. In comparison at one and seven months there was more pore space in the

bulk soil, and visualisation of individual aggregates within the macrocosms can be made.

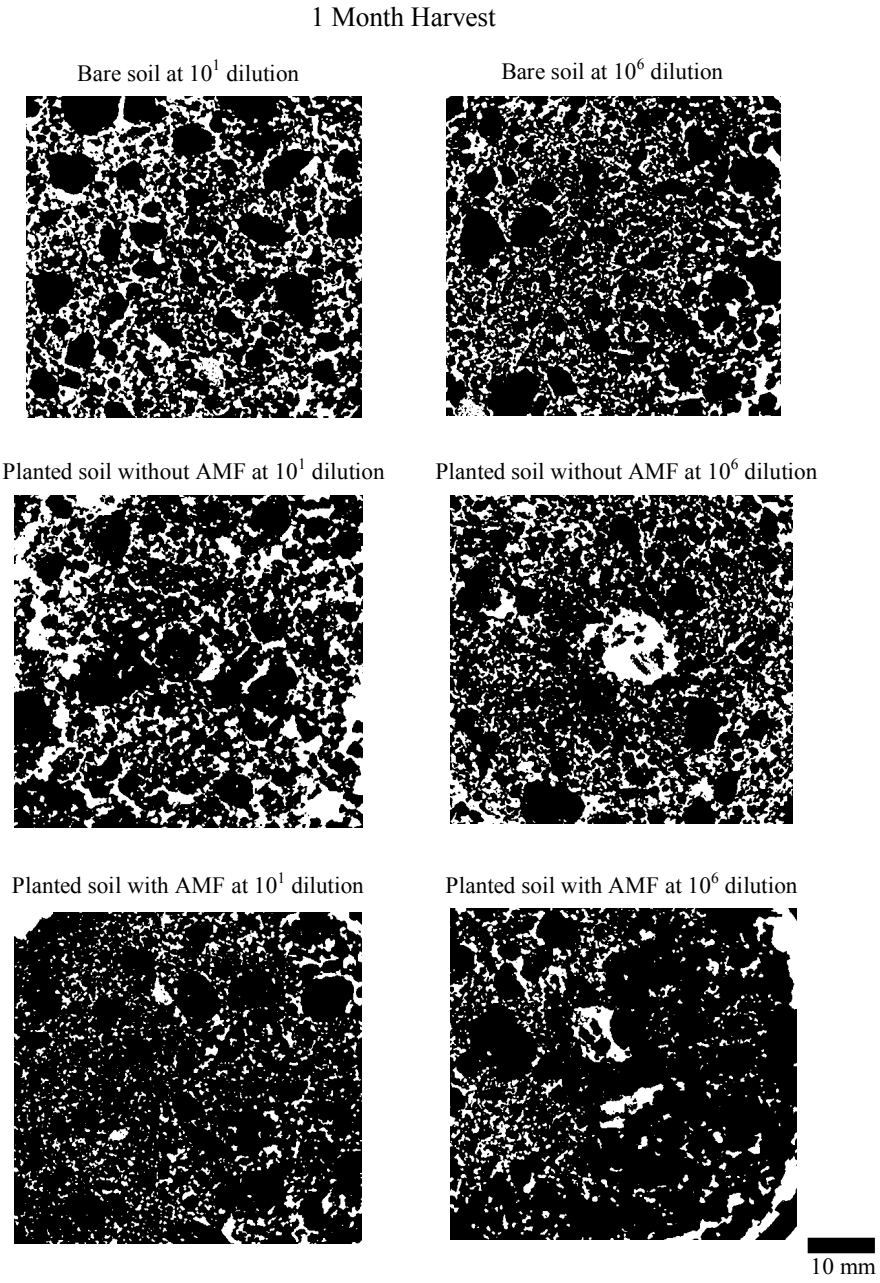


Figure 4.36: Representative images of soil structure at the first month harvest within all treatments.

Note: White represents pore space and black represents the soil matrix. One pixel = 65.4 μm.

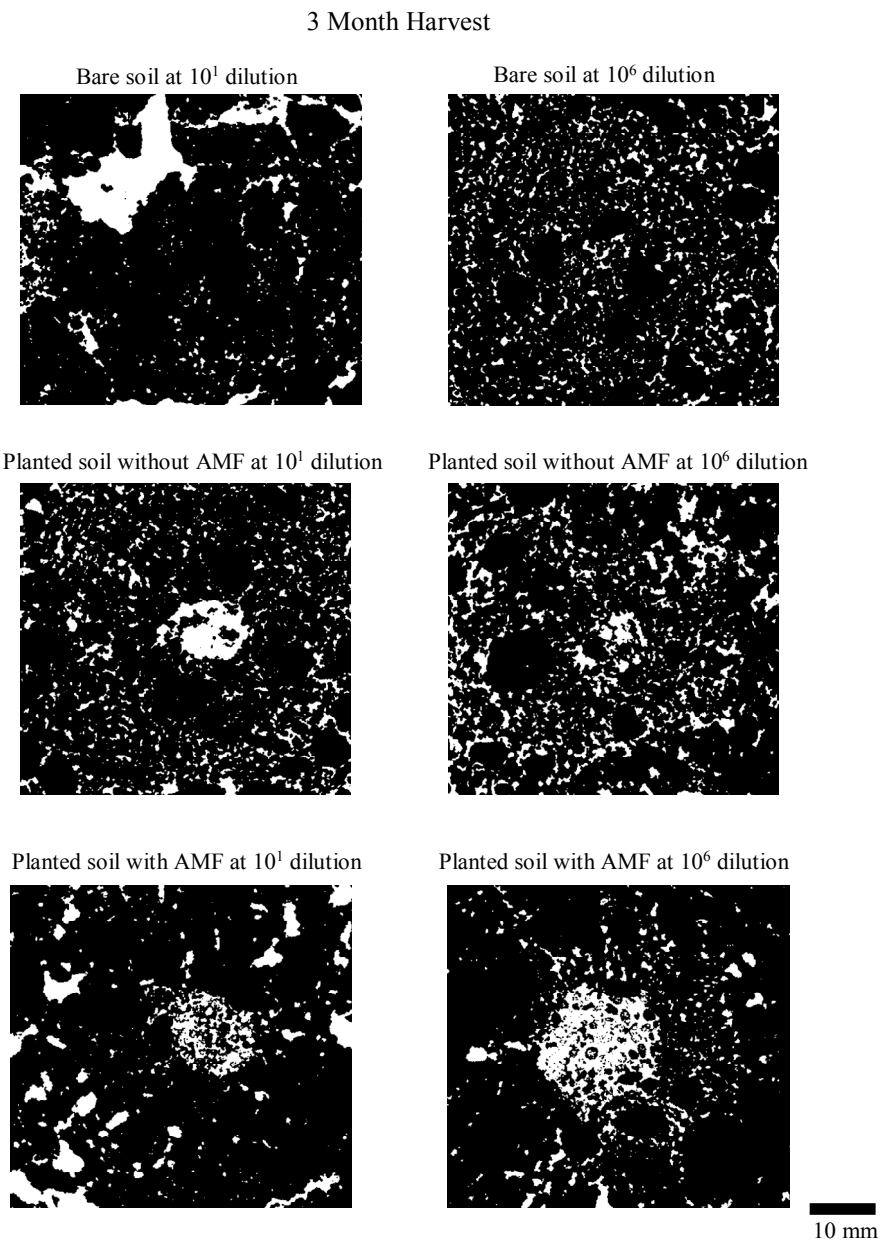


Figure 4.37: Representative images of soil structure at the third month harvest within all treatments.

Note: White represents pore space and black represents the soil matrix. One pixel = 65.4 μm.

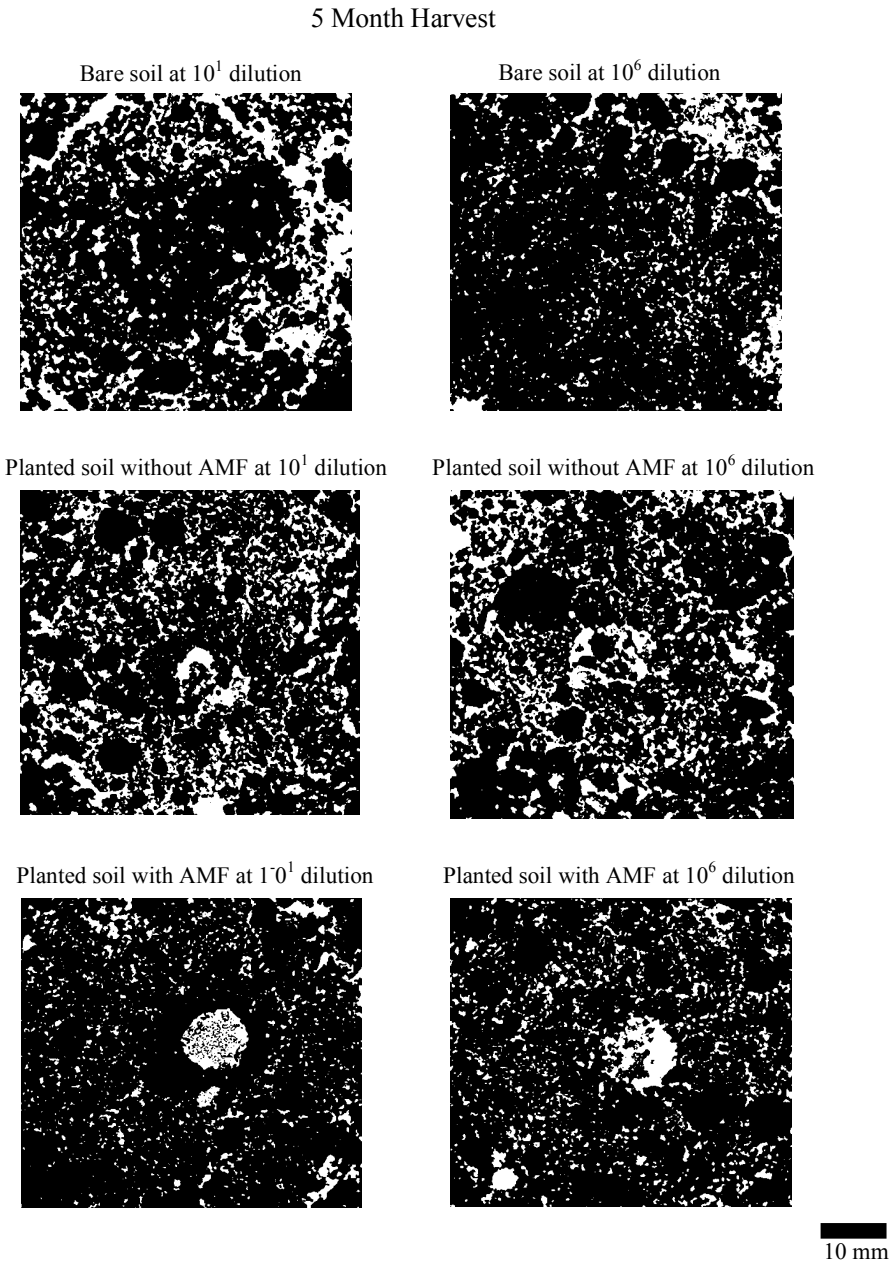


Figure 4.38: Representative images of soil structure at the fifth month harvest within all treatments.

Note: White represents pore space and black represents the soil matrix. One pixel = 65.4 μm.

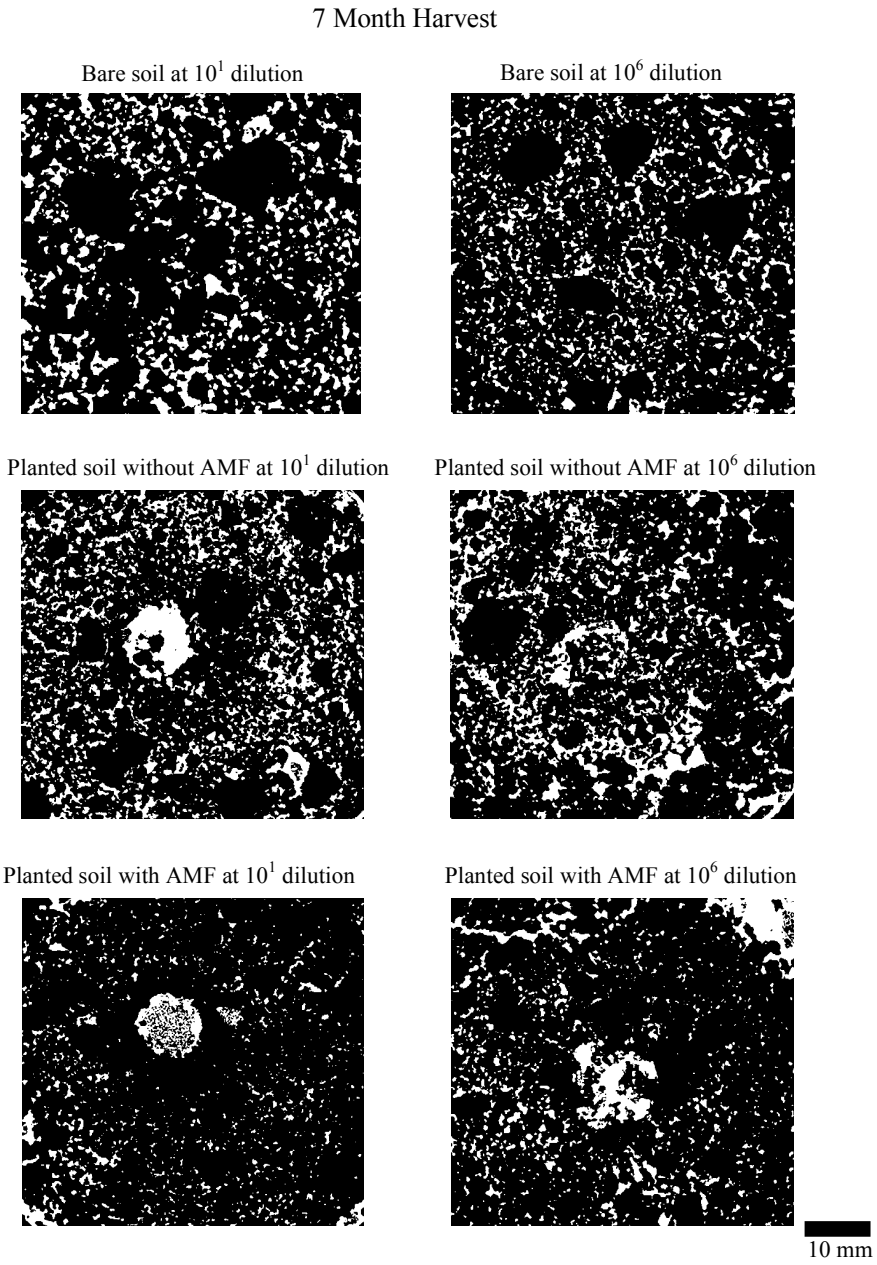


Figure 4.39: Representative images of soil structure at the fifth month harvest within all treatments.

Note: White represents pore space and black represents the soil matrix. One pixel = 65.4 μm.

Within the planted macrocosms (across all harvest except the first) the impact of *P. lanceolata* roots on porosity can clearly be observed by the large area of pore space in

the centre of the images. This entire area represents pore space (and to some extent root material due to the similarity in density of these objects determined from X-ray CT). Within the third month harvest, planted +AMF treatment (with the 10^6 dilution), individual roots can clearly be observed within this rhizospheric region.

4.3.12 TOTAL POROSITY

Quantitative assessment from X-ray CT images (where the smallest measurable pore was 65 µm in size), showed that various factors had an significant impact on total porosity throughout the macrocosms (Figure 4.40). Porosity was significantly lower ($F_{2,35}$ = 19.31, P < 0.001) within the planted +AMF treatment (12.5 %) and highest within the planted -AMF treatment (21.3 %). The bare soil treatment had a total porosity value of 18.1 %. Porosity was highest after one month (23 %) and dropped thereafter to 12, 18 and 16 % in months three, five and seven respectively (harvest effect, $F_{3,72}$ = 86.41, P < 0.001).

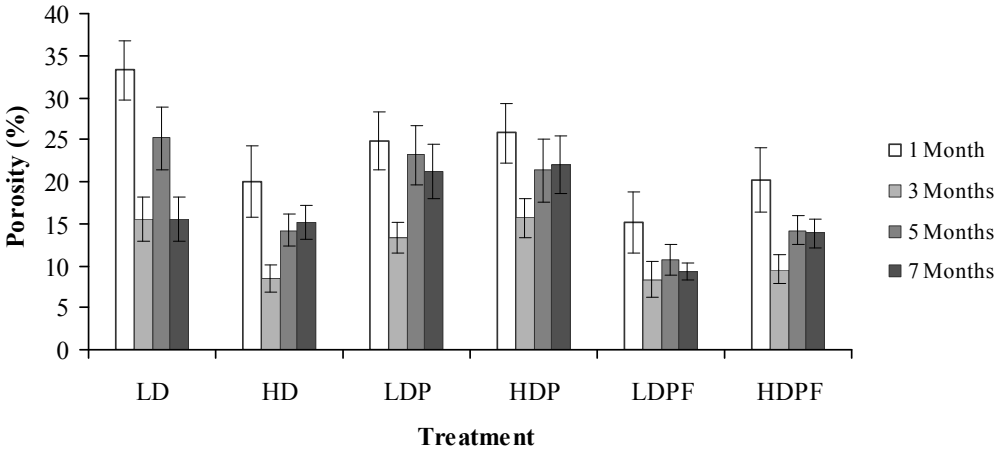


Figure 4.40: Total porosity of the soil within the macrocosms measured by X-ray CT at four harvest periods after inoculation and planting.

Data are means ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

Total porosity was also influenced by a dilution x treatment interaction, ($F_{2,35}$ = 6.93, P = 0.003) but only within the bare soil treatment where the 10^1 dilution had a higher total porosity compared to the 10^6 (21.7 % and 14.5 % respectively) (Table 4.12). No effect of dilution on total porosity was observed within the planted non-AMF treatment. Porosity within the planted AMF treatment was the lowest of all treatments and whilst the 10^6 soil dilution appeared to increase porosity in the later months (relative to the 10^1 treatment), this was not significant (Table 4.12).

Increases in porosity of up to 59 % were observed within the 10^1 dilution planted macrocosms, with different rates of increase observed within each treatment (Table 4.13).

Table 4.12: Mean total porosity under the two different dilution levels within the bare soil and planted macrocosms with and without AMF.

Data are means ± standard errors.

	Treatment		
Dilution level	**Bare soil treatment**	**Planted treatment with no AMF**	**Planted treatment with AMF**
10^1 (Low dilution)	21.7 ± 1.99	21.2 ± 1.77	10.6 ± 0.99
10^6 (High dilution)	14.5 ± 1.28	21.4 ± 1.83	14.4 ± 1.16

Table 4.13: Percentage change in porosity (calculated from mean values) after the initial soil settling period to the fifth and seventh month harvest periods (as measured from X-ray CT).

Data are means ± standard errors.

Treatment	**% change in porosity**	
	3-5 Months	**3-7 Months**
Bare soil (10^1)	61.61	0.24
Bare soil (10^6)	67.37	77.56
Planted -AMF (10^1)	73.92	59.13
Planted -AMF (10^6)	36.36	40.38
Planted +AMF (10^1)	28.82	11.08
Planted +AMF (10^6)	48.62	45.39

Porosity decreased with soil depth ($F_{5,35}$ = 21.25, P < 0.001), with a significant interaction of soil depth x treatment (i.e. the absence or presence of *P. lanceolata* with and without AMF) influencing total porosity within the macrocosms ($F_{10,35}$ = 3.30, P = 0.004; Table 4.14). Soil dilution amendment had no affect on the depth related reduction in total porosity.

Table 4.14: Total porosity (%) results for the soil macrocosms over each of the four harvest periods and with depth down the macrocosms.

Data are means ± standard errors.

	Depth from the top of soil surface (mm)					
Treatment	**35**	**55**	**75**	**95**	**115**	**135**
Bare soil	22.4 ± 3.28	28.6 ± 3.14	18.7 ± 1.79	19.2 ± 3.26	13.3 ± 1.72	6.7 ± 1.82
Planted without AMF	20.5 ± 1.30	39.0 ± 2.40	25.8 ± 1.31	25.4 ± 3.46	12.7 ± 1.98	8.9 ± 0.85
Planted with AMF	18.8 ± 1.33	15.4 ± 1.57	12.4 ± 1.74	10.1 ± 0.83	7.3 ± 0.70	9.8 ± 2.97

4.3.13 MEAN PORE SIZE

The smallest mean pore size (0.55 mm^2) was observed in the plants +AMF treatment compared to that of the bare soil (0.74 mm^2) and planted non-AMF (0.80 mm^2) treatments ($F_{2,35} = 27.55$, $P < 0.001$), suggesting that fungi reduced macropores. Mean pore size was highest at the first month, after which it generally decreased (harvest effect, $F_{2,72} = 13.96$, $P < 0.001$). The smallest pores were observed in the planted +AMF treatment in soils amended with the 10^1 dilution. This effect was lost in the 10^6 treated soils. A similar trend was observed in the planted -AMF treatment, although not as pronounced. A significant difference in pore size resulting from dilution amendment was observed in the bare soils (Figure 4.41; Table 4.15).

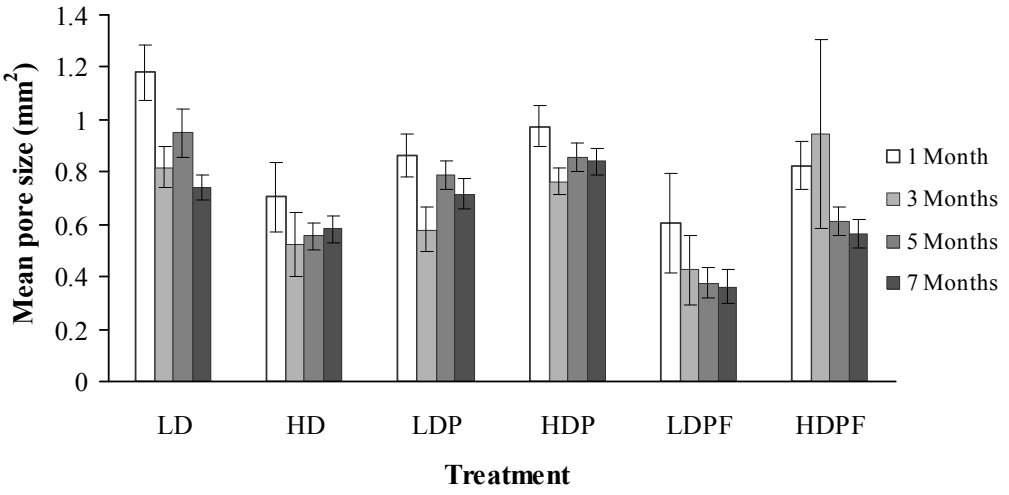


Figure 4.41: Mean pore size of the soil within the macrocosms measured by X-ray CT at four harvest periods after inoculation and planting.

Data are mean vales (log_{10}+1) ± standard error bars. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

Table 4.15: Mean pore size (log_{10} +1) under the two different dilution levels within the bare soil and planted macrocosms with and without AMF ($F_{2,35}$ = 44.41, P < 0.001).

Data are means ± standard errors.

Dilution level	Bare soil treatment (mm^2)	Planted treatment with no AMF (mm^2)	Planted treatment with AMF (mm^2)
10^1 (Low)	0.90 ± 0.05	0.74 ± 0.03	0.42 ± 0.05
10^6 (High)	0.58 ± 0.04	0.85 ± 0.03	0.69 ± 0.07

Pore size was reduced with increasing depth through the macrocosms. The smallest pores were observed in the mycorrhizal planted treatment from 55 mm downward (Table 4.16).

Table 4.16: Mean pore size (log_{10} +1) for each soil macrocosms treatment with depth down the macrocosms ($F_{10,35}$ = 4.71, P = 0.001).

Data are means ± standard errors.

	Depth from top of soil surface within macrocosm (mm)					
Treatment	**35**	**55**	**75**	**95**	**115**	**135**
Bare soil	1.01 ± 0.09	0.76 ± 0.08	0.71 ± 0.06	0.66 ± 0.08	0.64 ± 0.06	0.52 ± 0.06
Planted without AMF	0.78 ± 0.04	0.91 ± 0.05	0.94 ± 0.05	0.90 ± 0.04	0.68 ± 0.05	0.57 ± 0.04
Planted with AMF	0.99 ± 0.12	0.43 ± 0.05	0.50 ± 0.07	0.46 ± 0.03	0.40 ± 0.04	0.30 ± 0.05

4.3.14 PORE SIZE DISTRIBUTION

AMF inoculation generally resulted in an equal distribution of pore sizes in months one to three. After month five, differences in pore size distributions (PSD) were observed in AMF treated macrocosms (with more pores 1-3.16 mm^2 and 100-1000 mm^2 in size), whereas near normal distributions observed in other treatments (highlighting the dominance of pores 0.316-3.16 mm^2 in size). This is in contrast to the planted (but non-mycorrhizal) soils which show an almost normal pore size distribution (with total dominance of pores 1-10 mm^2 in size) in months five and seven with the 10^1 soil dilution, whereas distributions were skewed by the 10^6 dilution with more larger pores (100-1000 mm^2 in size). PSDs in bare soil generally followed similar patterns to those in the planted non-AMF macrocosms, particularly in soils amended with the 10^1 dilution. In bare soil, the PSD in both 10^1 and 10^6 amended soils was similar after seven months, but there are clear differences leading up to that point (Figure 4.42-4.46).

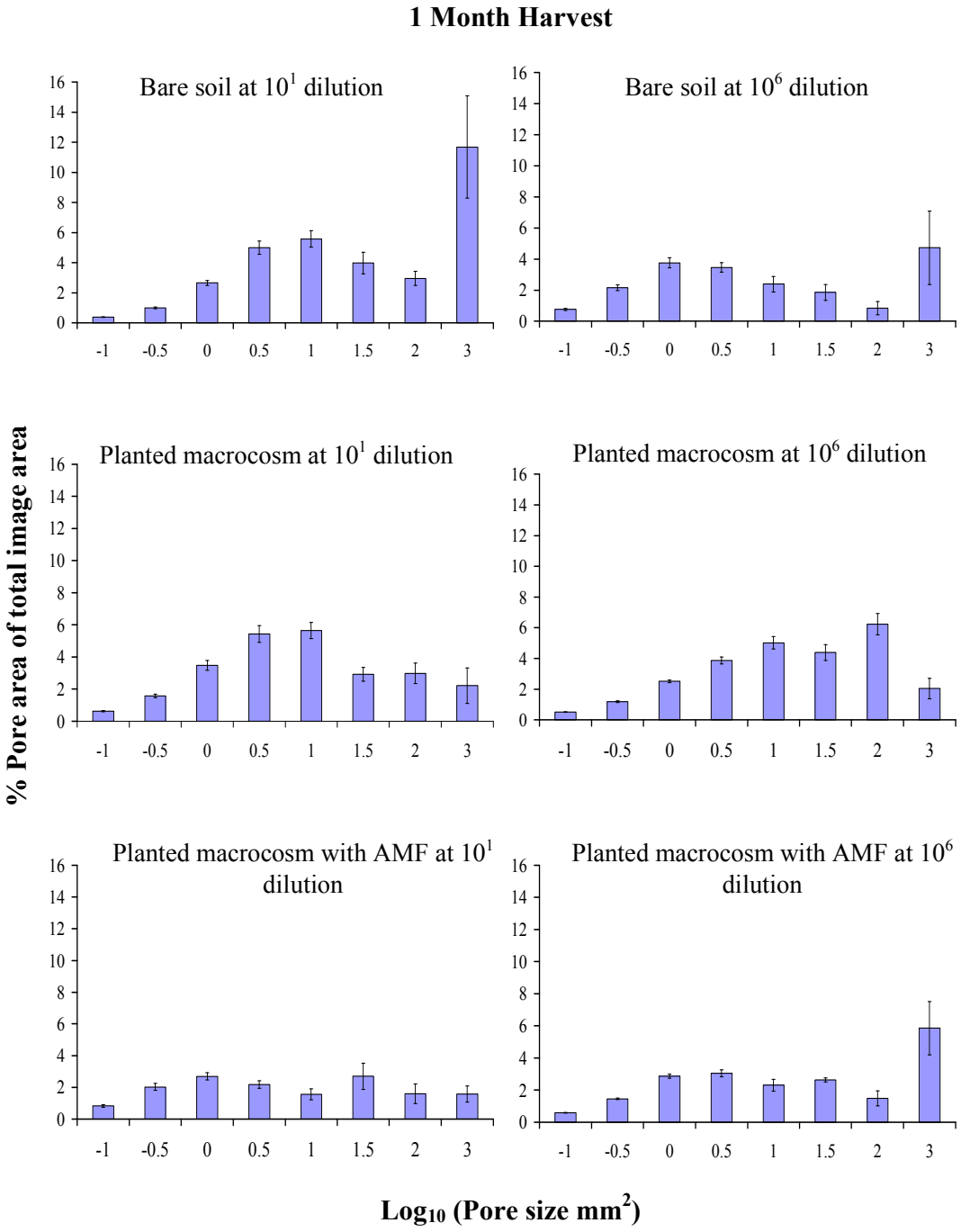


Figure 4.42: Pore size distribution for all treatments at the first (one month harvest) expressed as percentage of total image area.
Values are means ± standard error.

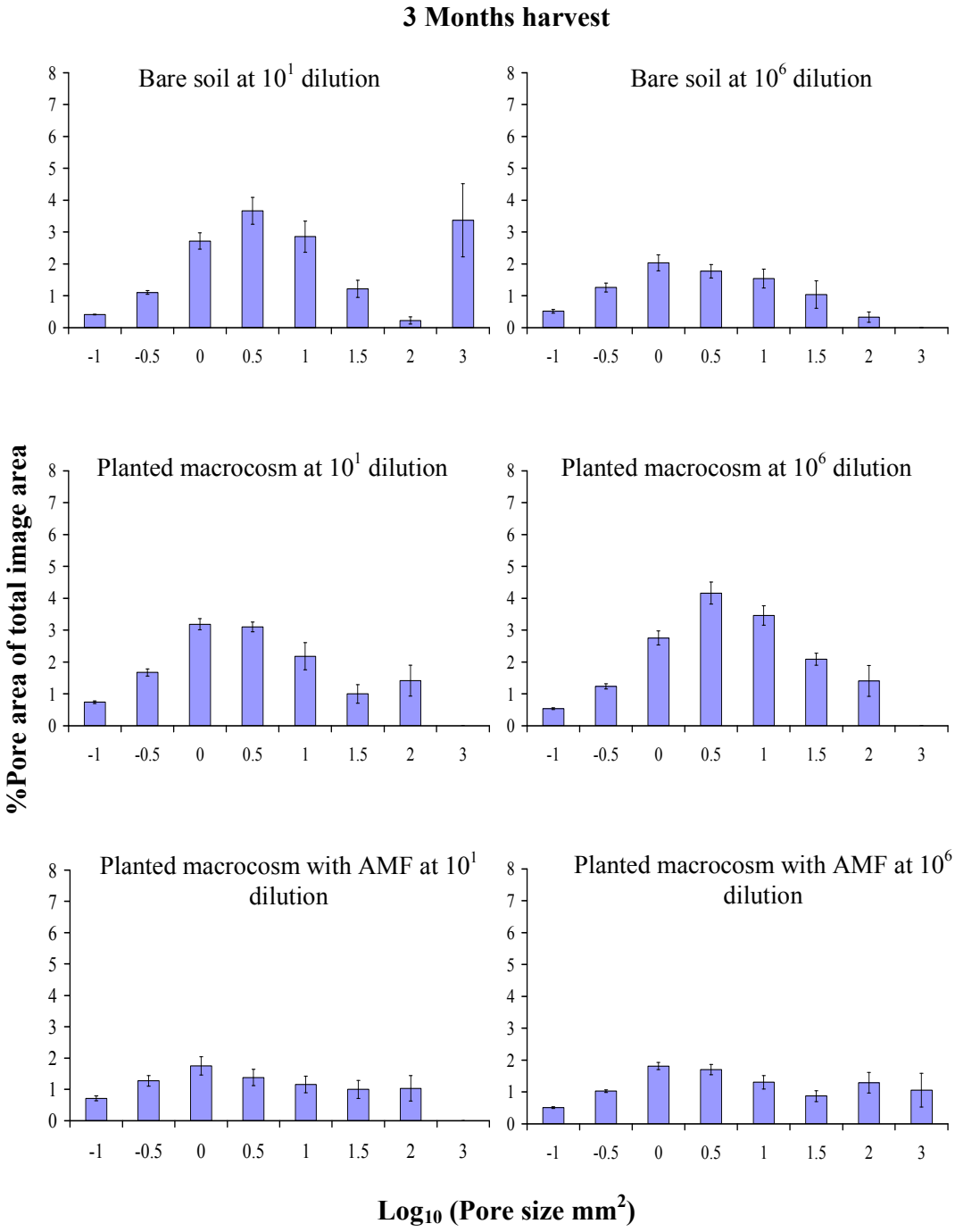


Figure 4.43: Pore size distribution for all treatments at the third month harvest expressed as percentage of total image area.
Values are means ± standard error.

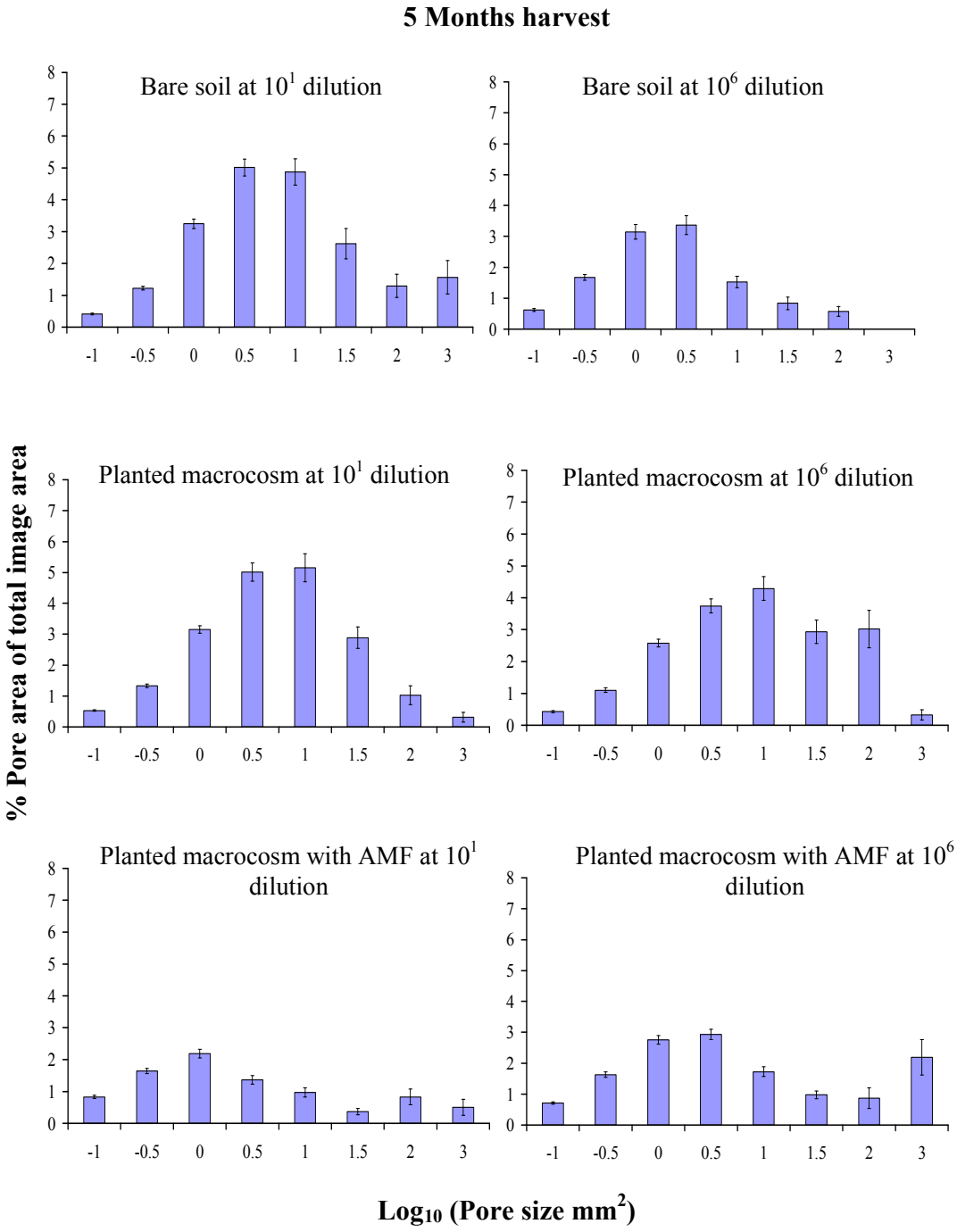


Figure 4.44: Pore size distribution for all treatments at the third month harvest expressed as percentage of total image area.
Values are means ± standard error.

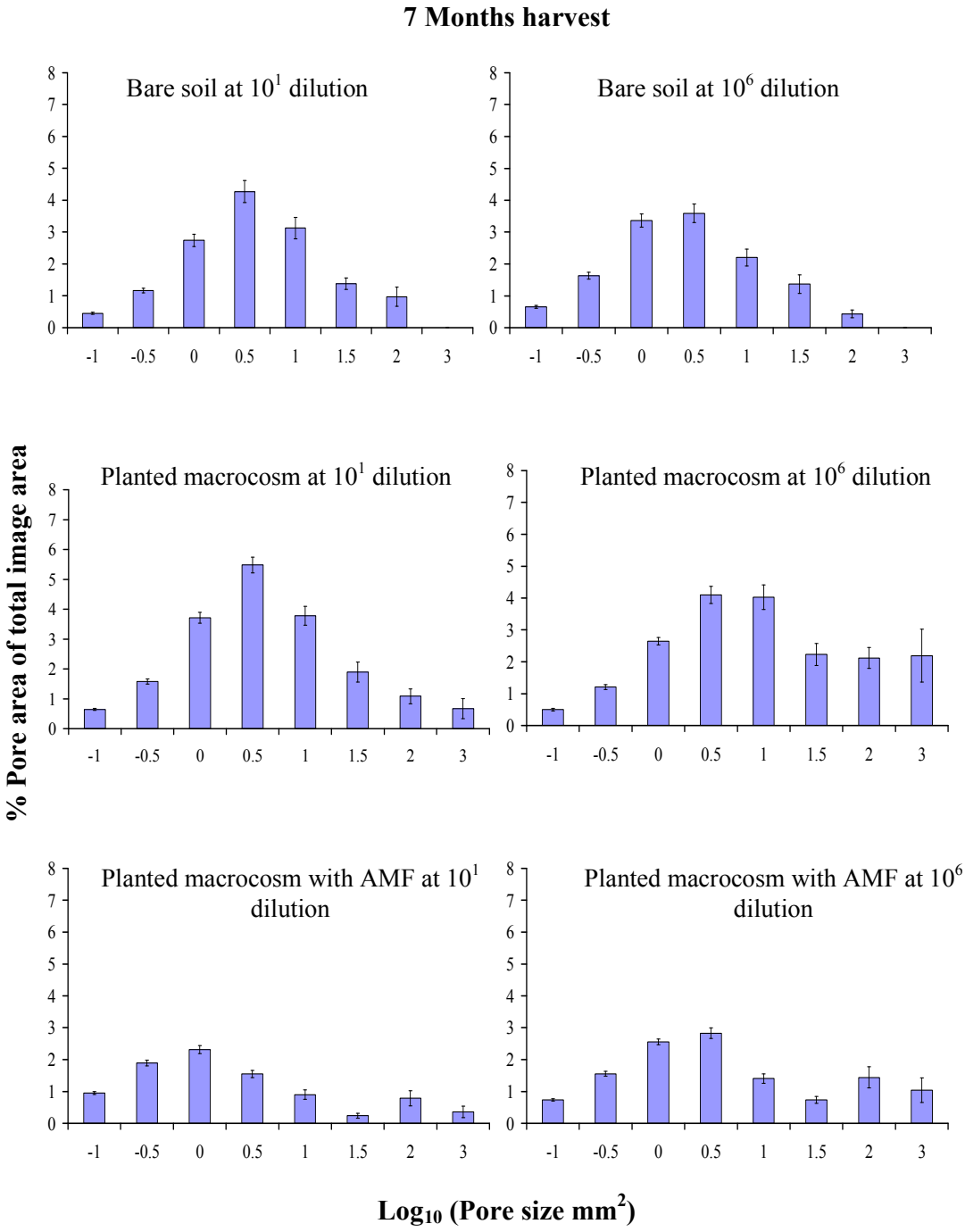


Figure 4.45: Pore size distribution for all treatments at the third month harvest expressed as percentage of total image area.
Values are means ± standard error.

4.3.15 PORE PERIMETER

Pore perimeter was significantly influenced by treatment, depth within the macrocosm (Figure 4.46) and harvest period (Table 4.17). Pore perimeter was highest within the planted non-AMF treatment (0.50 mm) compared to that of the bare soil (0.44 mm) and the planted AMF treatment, where pore perimeter was at its lowest (0.33 mm). Pore perimeter was highest at the first month harvest (0.53 mm) before significantly declining at 3 months (0.40 mm).

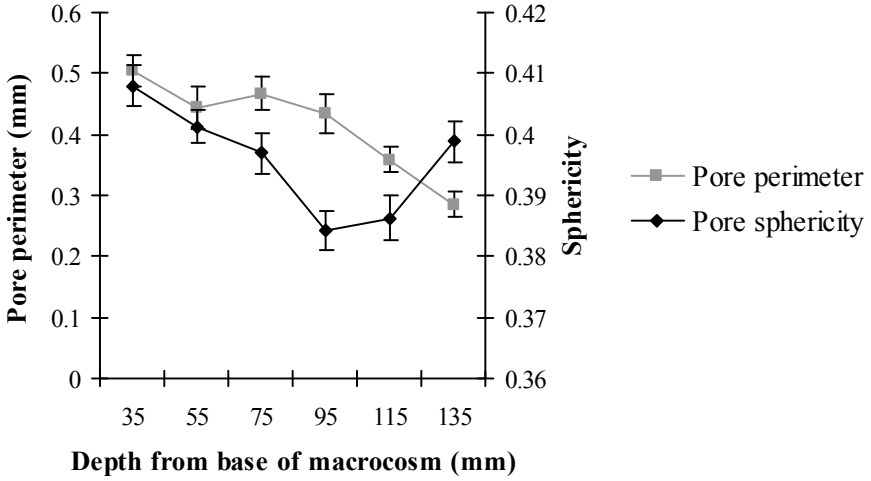


Figure 4.46: Effect of depth within the macrocosm on pore perimeter (log_{10} +1) and sphericity for all treatment and at all harvests.
Data are mean values ± standard error.

Within the bare soil treatment, pore perimeter had a higher value in the 10^1 dilution macrocosms (0.54 mm) compared to the 10^6 dilution (0.35 mm). The reverse however was true within the planted AMF macrocosms where pore perimeter was significantly lower (0.26 mm) in the 10^1 dilution macrocosms compared to the 10^6 dilution macrocosms (0.40 mm) (dilution x treatment interaction, $F_{2,35} = 35.52$, $P < 0.001$;

Figure 4.47). No significant difference in perimeter was observed in the planted non-AMF macrocosms despite the 10^1 dilution macrocosms having a smaller perimeter (0.48 mm) than at the 10^6 dilution level (0.52 mm). Furthermore there was a dilution x harvest interaction (Table 4.17) where the first and fifth month harvests had higher perimeter values within the 10^1 dilution macrocosms than the 10^6 dilution. See Table 4.17 for ANOVA results.

Table 4.17: Significant results from repeated measurement ANOVA for pore perimeter.

Source of variation	DF	F	P
Treatment	2	33.34	< 0.001
Depth	5	13.06	< 0.001
Dilution x Treatment	2	35.52	< 0.001
Treatment x Depth	10	3.70	0.002
Residual	*35*		
Harvest	3	23.73	< 0.001
Harvest x Dilution	3	5.02	< 0.019
Harvest x Treatment	6	4.56	0.008
Harvest x Depth	9	2.99	0.027
Harvest x Diversity x Treatment	6	2.63	0.065
Residual	*72*		

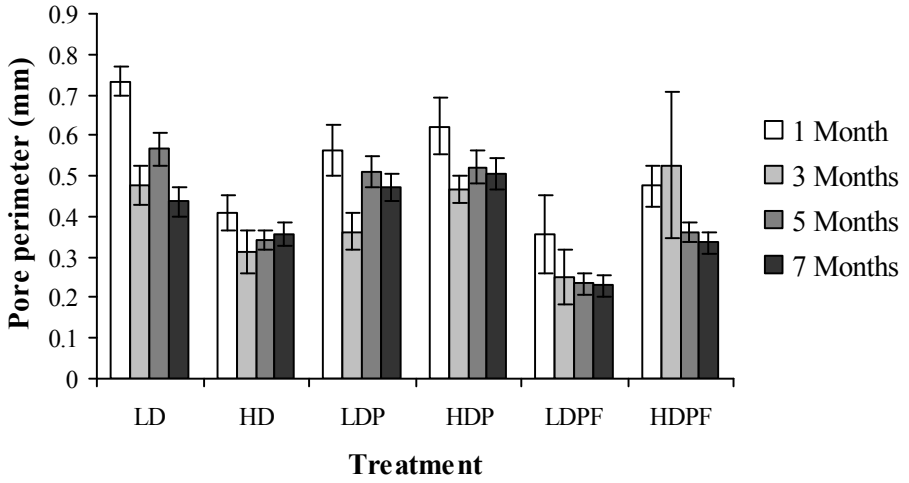


Figure 4.47: Pore perimeter determined from X-ray CT images for all treatments at each harvest period.

Data are means (log_{10}+1) ± standard error. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

4.3.16 PORE SPHERICITY

Pore sphericity decreased with increasing soil depth although this trend was reversed at depths greater than 95 mm from the surface (depth as single factor, Figure 4.46; a value of 1 signifies maximum circularity).

A treatment x dilution x harvest interaction was evident with the most noticeable differences being between mycorrhizal and non-mycorrhizal (planted) treatments. Pore spaces were more spherical in soils from planted mycorrhizal columns which had been amended with the 10^1 dilution compared to the 10^6 dilution. In contrast, soil dilution had no effect on pore sphericity in the non-mycorrhizal (planted) macrocosms. In the unplanted (bare) treatments, sphericity was more pronounced in columns amended with the 10^6 dilution than in those with the 10^1 dilution. Sphericity increased over the

duration of the experiment in the bare soil amended with the 10^1 dilution whilst it remained more constant over time in the other treatments, most notably within the planted mycorrhizal (planted) soils (Figure 4.48). See Table 4.18 for details of significant interactions.

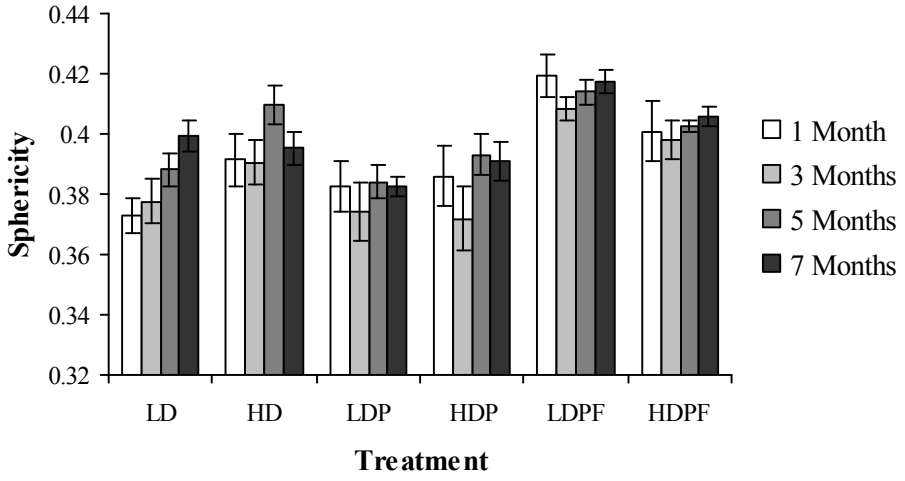


Figure 4.48: Pore sphericity determined from image analysis of X-ray CT images from each treatment at each harvest period.
Data are mean values ± standard errors. (Treatment codes LD and HD = 10^1 and 10^6 in a bare soil treatment; LDP and HDP = 10^1 and 10^6 dilution in a planted non-AMF treatment respectively, LDPF and HDPF = 10^1 and 10^6 dilution in a planted AMF treatment).

Table 4.18: Results from repeated measurement ANOVA for pore sphericity.

Source of variation	DF	F	P
Treatment	2	25.12	< 0.001
Depth	5	6.80	< 0.001
Dilution x Treatment	2	5.77	0.007
Residual	*35*		
Harvest	3	17.23	< 0.001
Harvest x Treatment	6	4.08	0.002
Harvest x Dilution x Treatment	6	2.48	0.038
Residual	*72*		

4.3.17 NEAREST NEIGHBOUR DISTANCE

The distance between pores (nearest neighbour distance) became greater with increasing depth down the column. For example at a depth of 55 and 135 mm, nearest neighbour distances were 0.97 and 1.08 mm (log_{10}+1 transformed) respectively ($F_{5,35}$ = 6.51, P < 0.01). From the first to the third month harvest the near neighbour distance between pores increased from 1.02 to 1.08 mm (log_{10}+1 transformed) respectively. Thereafter, distances were reduced to 1.04 and 1.02 mm at the fifth and seventh month (harvest effect, $F_{3,72}$ = 7.65, P = 0.008).

In planted soils, pores were nearer to each other than in bare (unplanted) soil when the treatments had been amended with the 10^1 dilution (Figure 4.51). This effect was not apparent in soils amended with the 10^6 dilution (treatment x dilution interaction, $F_{2,35}$ = 7.32, P = 0.002). Mycorrhizal status had no effect on nearest neighbour distances.

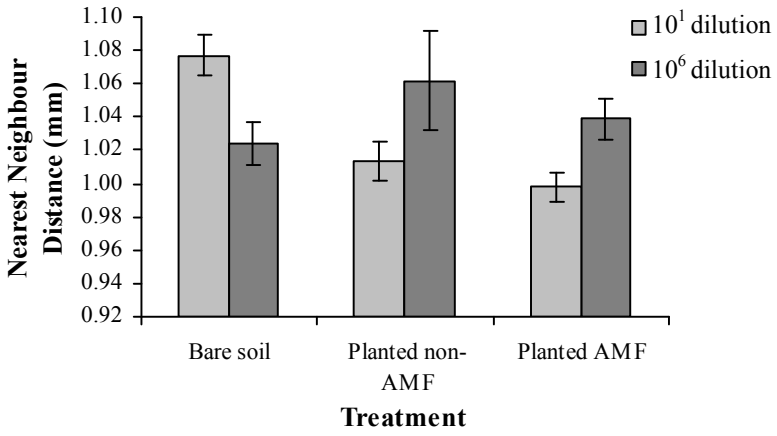


Figure 4.49: Nearest neighbour distance between pores located within each dilution and treatment at all harvest periods.
Data are mean values (log_{10} +1) ± standard errors.

4.3.18 LINKING SOIL PHYSICAL PROPERTIES WITH SOIL MICROBIAL MEASUREMENTS.

A Pearson's product moment correlation was undertaken to identify correlations within the data set generated over the course of this experiment. Several significant correlations were observed (Table 4.19) and regression analyses performed on the more important relationships (described below).

A significant positive relationship was observed between percentage root length colonised by AM fungi and total substrate utilisation within Biolog microtitre plates after 72 hours incubation (Figure 4.50) and after 96 hours (data not shown). Within the planted macrocosms (both dilutions combined) microbial biomass-C increased in tandem with root biomass (Figure 4.51).

Table 4.19: Results from a Pearson's product moment correlation showing the significance level of interaction between selected measured factors in additon to the type of relationship (i.e. + = positive, - = negative).

NB: Shaded boxes are significant relationships. Critical values 0.404, 0.472 and 0.515 when P is = 0.05; 0.02 and 0.01 respectively.

	Root biomass	Shoot biomass	AMF colonisat-ion	Organic Matter	Total utilisation at 72hrs	Total utilisation at 96hrs	Soil biomass	Bacterial species richness	Fungal species richness	Aggregate stability	Total Porosity	Mean pore size	Pore perimeter
Shoot biomass	+ 0.8498 (P < 0.01)												
Total utilisation at 72hrs	+ 0.5019 (P < 0.02)	+ 0.5489 (P < 0.01)	+ 0.6499 (P < 0.01)	-0.2941									
Total utilisation at 96hrs	+ 0.4626 (P < 0.05)	+ 0.5243 (P < 0.01)	+ 0.6413 (P < 0.01)	-0.3337	+ 0.9819 (P < 0.01)								
Soil biomass	+ 0.7803 (P < 0.01)	+ 0.5311 (P < 0.01)	+ 0.1776	-0.1881	+ 0.3418	+ 0.2846							
Fungal species richness	+ 0.2541	+ 0.2853	+0.1996	-0.1157	+ 0.4568 (P < 0.05)	+ 0.3913	+ 0.161	+ 0.3349					
Fungal species diversity	+ 0.1437	+ 0.0713	+ 0.2856	- 0.4367 (P < 0.05)	+ 0.4482 (P < 0.05)	+ 0.4093 (P < 0.05)	+ 0.1373	+ 0.2575	+ 0.7391 (P < 0.01)				
ASDcu	-0.1927	-0.3094	-0.2147	-0.1989	- 0.4742 (P < 0.02)	- 0.4677 (P < 0.05)	-0.0269	-0.0238	+ 0.0263				
Aggregate stability	- 0.5015 (P < 0.02)	-0.0889	-0.1329	+ 0.5582 (P< 0.01)	-0.0463	-0.0464	- 0.5774 (P < 0.01)	+ 0.3026	-0.0435				
R index	+ 0.621 (P < 0.01)	+ 0.4947 (P < 0.02)	+ 0.308	-0.3263	+0.1763	+ 0.2355	+ 0.2894	-0.2314	-0.1413	- 0.4394 (P < 0.05)			
Total Porosity	-0.0711	-0.1959	- 0.5236 (P < 0.01)	-0.2867	-0.1864	-0.1497	-0.0031	+ 0.6007 (P < 0.01)	-0.0334	-0.1548			
Mean pore size	-0.2146	-0.1382	- 0.5439 (P < 0.001)	+0.0061	-0.1693	-0.1134	-0.2666	+0.3561	-0.1837	+0.1382	+ 0.8095 (P < 0.01)		
Pore perimeter	-0.1563	-0.1044	- 0.5771 (P < 0.01)	-0.0294	-0.1777	-0.1275	-0.1985	+ 0.4244 (P < 0.05)	-0.148	+0.0911	+ 0.8674 (P< 0.01)	+ 0.9865 (P < 0.01)	
Sphericity	+ 0.0656	-0.0485	+ 0.7295 (P < 0.01)	- 0.4128 (P < 0.05)	+ 0.2282	+ 0.2259	+ 0.0023	-0.3568	+0.1877	-0.268	- 0.5541 (P < 0.01)	- 0.6334 (P < 0.01)	- 0.6747 (P < 0.01)
Nearest neighbour diameter	-0.2207	+ 0.0397	-0.2514	+ 0.4489 (P < 0.05)	-0.1446	-0.0944	-0.394	-0.3099	-0.253	+0.2692	-0.0343	+ 0.5073 (P < 0.02)	+ 0.4173 (P < 0.05)

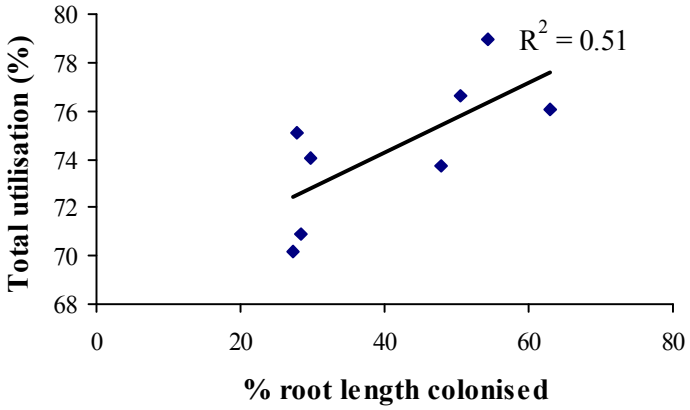


Figure 4.50: Relationship between per cent root length colonised by AM fungi and total substrate utilisation within Biolog plates measured after 72 hours incubation for all soil macrocosms containing AMF; all harvest periods combined (Regression, P = 0.046). ***AMF data are arcsin square root transformed.***

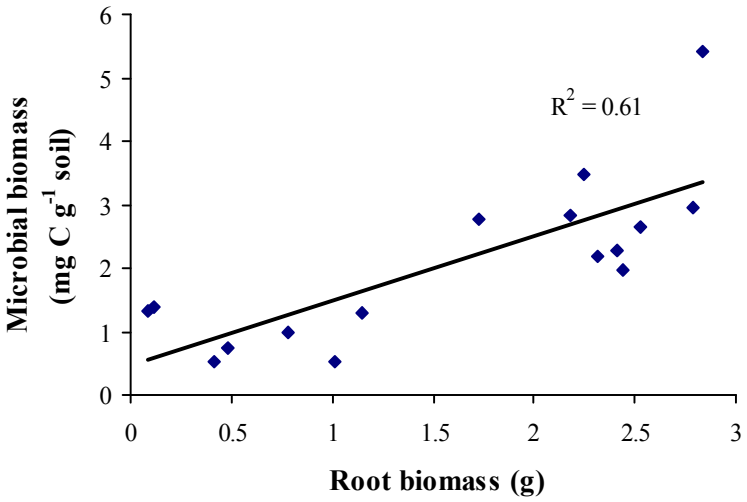


Figure 4.51: Relationship between microbial-C and root biomass for each planted soil treatment; all harvest periods combined (Regression, P < 0.001).

Moreover soil microbial measurements were also significantly correlated with soil structure properties. Microbial biomass had a significant negative impact on aggregate stability; as microbial biomass increased, aggregate stability declined (Figure 4.52A).

Aggregate stability was also negatively affected by increasing root biomass (Figure 4.52B); however, aggregate stability increased with increasing soil organic matter (Figure 4.53).

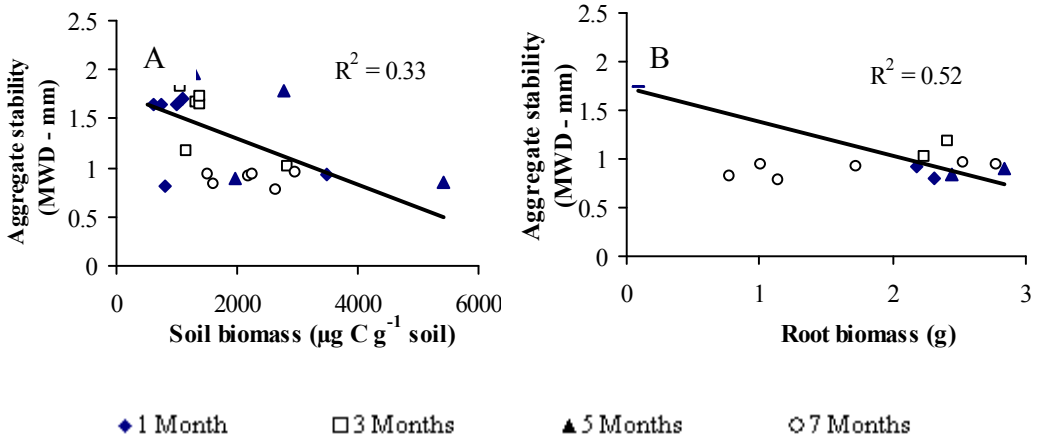


Figure 4.52: Relationship between A) aggregate stability and microbial biomass (Regression; P = 0.003) and B) aggregate stability and root biomass (square root transformed) (Regression, P < 0.001). All treatments and harvest periods were combined for analysis, but individual harvest periods are identified.

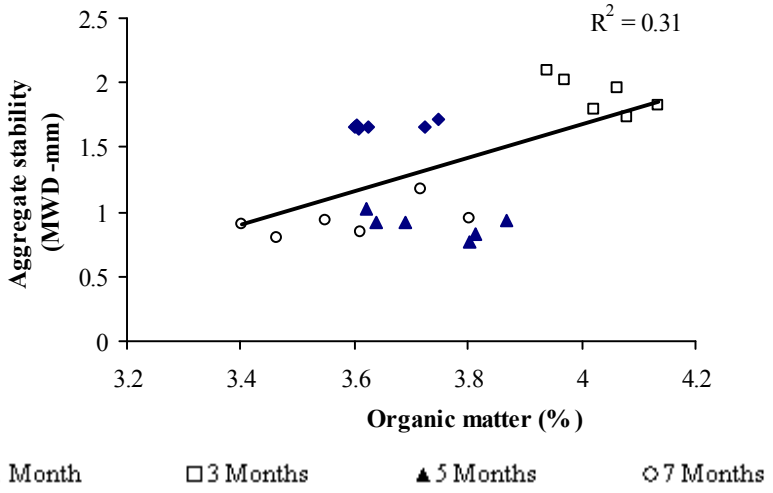


Figure 4.53: Relationship between aggregate stability and organic matter. Soil treatments and harvest periods combined for analysis (Regression, P = 0.005).

A significant relationship between bacterial species richness and porosity within all the soil macrocosms regardless of planting treatment was observed (Figure 4.54). Thus as bacterial species richness increased there was a corresponding increase in porosity.

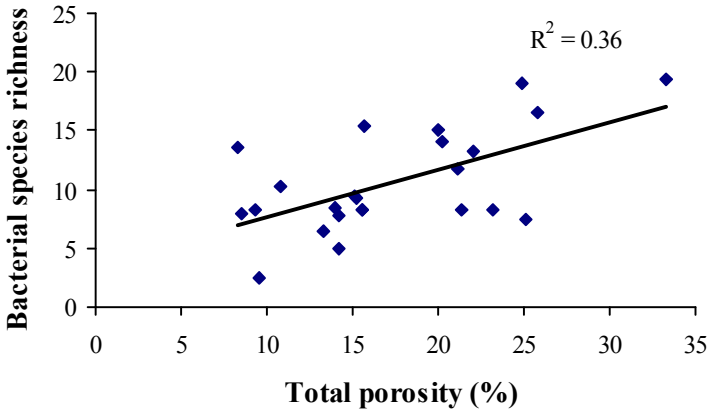


Figure 4.54: Relationship between bacterial species richness and total soil porosity for each soil treatment; all harvest periods combined (Regression, P = 0.003).

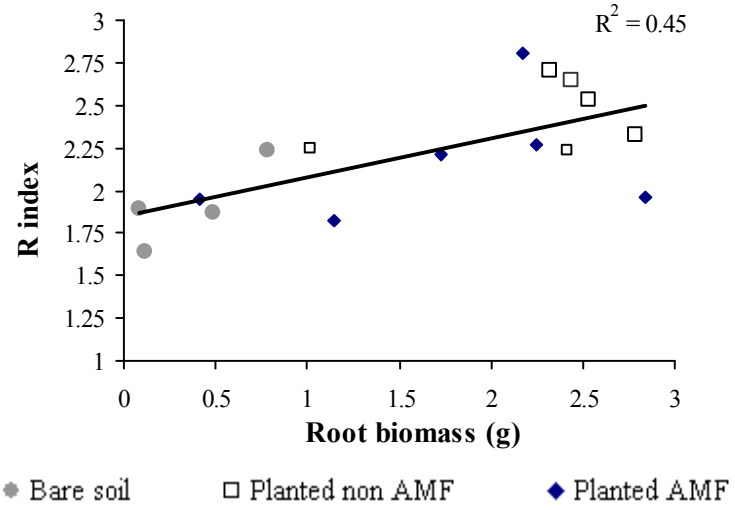


Figure 4.55: Relationship between water repellency index and root biomass (square root transformed); all treatments and harvests combined (Regression, P = 0.005).

An important correlation between root biomass and aggregate water repellency was also observed (Figure 4.55). As root biomass increased, water repellency (expressed as the repellency index) also increased.

Additional significant correlations ($P < 0.05$) were observed with measurements taken from image data (Figure 4.56). A significant relationship was observed between mean pore area and sphericity. As mean pore size increases the sphericity of the pore declines (Figure 4.56B). This suggests that as a pore become larger its shape changes, becoming more elongated than circular. However this relationship may not be wholly causal since pixel number influences sphericity. Smaller pores appear more rounded because fewer pixels make a pore within an image; compared to a larger pore, that comprises of more pixels. A similar relationship was also observed between pore perimeter and pore sphericity (Figure 4.56A). Other relationships such as the link between pore perimeter with mean pore area (Kampichler and Haser, 1993; Pachepsky *et al*., 1996; Figure 4.57) (and also total porosity), in addition to increase in distance between pores as pore area increases, were further observed (Table 4.19).

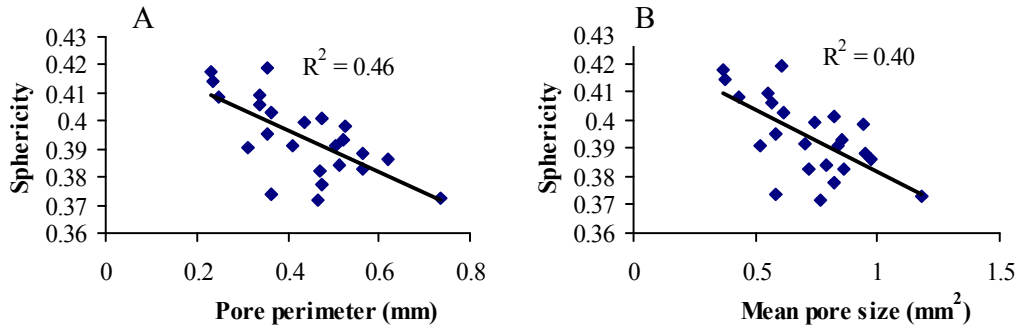


Figure 4.56: Relationship between pore sphericity and A) pore perimeter (log_{10} +1 transformed) (Regression, $P < 0.001$) and B) mean pore size (log_{10}+1) (Regression, $P < 0.001$). All treatments and harvests combined.

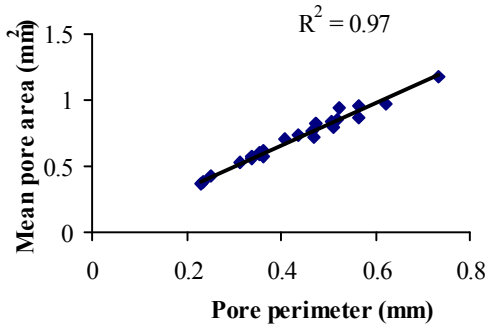


Figure 4.57: Relationship between pore perimeter and mean pore size (log_{10} +1) (Regression, P < 0.001). All treatments and harvests combined.

4.4 DISCUSSION

4.4.1 EFFECT OF MACROCOSM TREATMENT ON BIOLOGICAL PROPERTIES AND SOIL STRUCTURE

Total shoot and root biomass was influenced by treatment, particularly mycorrhizal fungal presence, with shoot and root dry weights highest within the planted non-AMF treatment. At the first month harvest in particular, shoot and root biomass was significantly lower in the mycorrhizal treated *P. lanceolata*. Such relationships were reported by van der Heijden *et al. (*2006), where shoot biomass of grassland species was lower in mycorrhizal plants after a second growing season with root biomass 26 % higher in non-AMF soil. After one month incubation *P. lanceolata* roots were highly colonised (~ 30 %), agreeing with previous research by Staddon, Graves and Fitter (1998) and Šmilauerová and Šmilauer, (2002) who found colonisation rates, of around 50 % within *P. lanceolata*. These relatively high colonisation rates, particularly after the one month harvest, were likely to be too high, resulting in the AMF becoming

parasitic in nature and being a drain on the plant's photosynthates (Johnson, Graham and Smith, 1997). This hypothesis was substantiated by mycorrhizal dependency values, which highlighted the reduced growth of *P. lanceolata* at the first harvest.

Furthermore, it is widely acknowledged that there is a tendency for plant species with thick coarsely branched roots to be more heavily mycorrhizal than species with thin roots (Baylis, 1975) which in turn have a lower mycorrhizal dependency (Eisssenstat, 1992). This is because thin roots have a larger surface area to volume ratio, allowing improved nutrient absorption and hence reducing the need for symbiosis with mycorrhizal fungi. Thus another explanation for the reduced shoot and root biomass within the AMF treatments could be due to *P. lanceolata* having thin roots, which meant the plant did not need to invest a large quantity of carbon to AMF. However, since the AMF inoculum was so efficient in colonising the roots it caused a drain in the seedling/young plant's carbon resources, reducing root and shoot growth (Collier, Yarnes and Herman, 2003).

The reduced quantity of root material within the AMF treated soil columns (yet similar shoot biomass) after three months, suggests that the mycorrhiza became mutualistic and *P. lanceolata* maintained itself despite a smaller root system. Alternatively, *P. lanceolata* had insufficient photosynthates to generate new root material (Eissenstat *et al*., 1993), which energetically is more costly than maintaining the AMF. The current data corroborate other studies which showed root biomass to be lower within mycorrhizal systems (Schubert and Lubraco, 2000); however results vary with plant

species and soil type studied, with evidence in some cases of AMF-induced increases in root biomass (van der Heijden *et al.,* 1998a; Cruz *et al.,* 2004; Copetta, Lingua and Berta, 2006). Changes in root morphology also occur in response to mycorrhizal colonisation with root diameter decreasing (Yao, Wang and Chen, 2009). This in turn could influence root exudate release, the stresses on soil by root movement and thus soil structure. The effects of root morphology on root exudation was discussed by Filho *et al.* (2004) who suggested that thin roots release more exudates from their extremities than thicker roots.

In the current investigation, soil biomass, total substrate utilisation and fungal species richness were all lowest within the bare soil environment, highlighting the importance of root activity on microbial properties within the soil (Grayston *et al.*, 1998). With the presence of AMF within the macrocosm, total substrate utilisation was highest. In the planted non-AMF treatment, soil biomass and species richness of bacteria were significantly higher than in other treatments. Interestingly, fungal species richness was similar in planted non-mycorrhizal and mycorrhizal treatments. These results highlight the importance of roots and their exudates on microbial properties and numbers within the soil (Jaeger *et al*., 1999; Walker *et al.,* 2003; Nappipieri *et al*., 2008). Whilst fungal species richness may have been similar irrespective of mycorrhizal status of the plants, total community DNA extracts showed that treatment influenced microbial community composition within the soil macrocosms. Three unique bacterial TRFs were found in the AMF-inoculated columns and just one unique fungal TRF. There were particular differences between the bare soil and planted macrocosms corroborating work by

Baudoin, Benizri and Guckert (2002), Marschner and Baumann (2003) and Remenant, Grundmann and Jocteur-Monrozier (2009).

It might be expected that mycorrhizal roots should enhance species richness to a greater extent than non-mycorrhizal roots since it is accepted that AMF exert selective pressures on microbial communities and increase root exudation (Fracchia *et al*., 1998; Johansson, Paul and Finlay, 2004). This was not the case here for fungi and it might be concluded that root biomass is of greater importance in this context than AMF *per se*. However, carbon substrate utilisation measured by Biolog GN2 technology (an indication of bacterial metabolic potential) was enhanced in mycorrhizal treatments compared to planted non-mycorrhizal soils. Grayston *et al*. (2001) stated that Biolog profiles may reflect carbon source availability, thus providing indirect evidence that AMF colonisation altered patterns of root exudation, possibly by changing carbon allocation in the plant. Alternatively, the three unique bacterial TRFs may have contributed to this finding.

With regard to the soil structure, the bare soil system, contained larger aggregates (hence a larger ASD_{CU}) than the planted non-AMF treatment and planted AMF treatment (that contained the smallest aggregates). The same pattern was also observed for mean pore area, where pores within the bare soil were larger than those in the planted-AMF macrocosm. This was due to roots within the planted macrocosms utilising pore space (particularly > 10 μm in diameter; that were introduced by packing) and compressing the surrounding soil (Dexter, 1987; Braunack and Freebairn,

1988) reducing pore area, total porosity (Brund *et al*., 1996) and fragmenting micro-aggregates that would ultimately form macro-aggregates (Tisdall and Oades, 1982; Angers and Coran, 1998).

Generally total porosity and pore perimeter (regardless of dilution level) was significantly higher in the planted non-AMF treatment which correlates with higher root biomass. This highlights the significance roots have on soil structure. The importance of roots on pore development was highlighted by White (2006) who suggested pores from 0.2-5000 μm in size are formed by root hairs, fine lateral roots and main roots. This relationship between porosity and root biomass was not observed within the planted +AMF macrocosms, where AMF colonisation may have decreased root thickness (Yao, Wang and Chen, 2009) and influenced microbial populations and their functioning within the rhizosphere. The mycorrhizal plants produced less root biomass than non-mycorrhizal plants which may have directly affect porosity. These findings contradict hypothesis three (section 1.5) and form the basis of a more detailed investigation in Chapter five.

4.4.2 Effect of Harvest Period on Biological Properties and Soil Structure

Root biomass and AMF colonisation, increased over the first five months. As the roots and mycorrhizal fungi, (where present) utilised nutrients closest to the root these reserves were depleted, forcing the root system to expand in search of new reserves and eventually causing the macrocosms to become root bound by the seventh month.

At the start of the investigation biomass and total substrate utilisation was at its lowest, suggesting that insufficient time was allowed for the added soil dilutions to reach equilibrium within the macrocosms, despite the highest bacterial species richness and diversity observed. Once root development and stabilisation of microbial communities had taken place within the macrocosm, soil biomass and total utilisation reached a peak in the fifth and seventh month respectively. Fungal and bacterial T-RFLP profiles of soil microbial communities differed over the harvest periods, with different unique fungal and bacterial fragments present at each of the different harvest periods. A possible explanation for this change in microbial community over time maybe because despite macrocosms being watered with sterilised water, they were not maintained in a sterile environment, so as Griffiths *et al.* (2001) highlighted there was a possibility of contamination from the surrounding environment over time, influencing the microbial populations present. In addition, as the microbial communities reached an equilibrium over time, species shifts would occur since some species would replace others as the most dominant in the system (and therefore most likely to be isolated).

In addition to biological changes taking place within the macrocosms over time porosity, mean pore area, pore perimeter and nearest neighbour distance were also influenced by harvest period. At the first harvest, porosity, mean pore size and pore perimeter was highest (and the distance between individual pores was lowest). A significant decrease was observed at the third month harvest with porosity, pore area, pore perimeter and ASD_{CU} declining to their lowest point (and the distance between pore spaces being significantly greater). These observations were due to settling of the soil particles (Leij, Ghezzehei and Or, 2002) after soil packing due to gravity and the

watering regime causing compaction. Porosity, mean pore size, pore perimeter and ASD_{CU} then increased (with a decline in distance between individual pore spaces also observed) in the fifth month suggesting signs of soil structure development. A decline in mean pore size and total porosity at the seventh month harvest was due to soil macrocosms becoming root bound, causing the roots to compact the available pore space as they grew and spread through the soil (Brund *et al.,* 1996). Braunack and Freebairn (1988) discovered using radiograph techniques that root elongation resulted in a 36 % increase in soil density at the root edge; with such density changes, significant compaction of the soil within the macrocosms analysed here would explain the decline in porosity and mean pore size. Reduction in ASD_{CU} at the final harvest also reflected the physical impact roots have on aggregates, with root activity known to cause aggregate breakdown (Caron, Kay and Perfect, 1992; Reid and Goss, 2006).

Patterns in aggregate stability by the third month were as expected, with aggregate stability highest within the planted +AMF treatment possibly because of the actions glomalin and other related substances (Wright and Upadhyaya 1996, 1998; Rillig, Wright and Eviner, 2002; Rillig, 2004); followed by aggregates stabilised by rhizospheric microbes (Czarnes *et al.,* 2000) and root exudates (Morel *et al*., 1991; Czarnes *et al*., 2000) and then least stable within the bare soil environment. This pattern was then lost when aggregate stability drastically decreased at the fifth month (coinciding with the highest level of water repellency seen within the aggregates). The peak in repellency took place when root biomass, AMF colonisation and soil biomass was significantly higher than in the previous harvest periods. Previous work by Hallett,

Gordon and Bengough (2003), also found water repellency higher within rhizosphere soil. This perhaps suggests that the chemicals, such as glomalin released by AMF and exudates released from microbe functioning, increased the water repellency of soils (Wallis and Horne, 1992; Hallett and Young, 1999; Czarnes *et al*., 2000; De Bano, 2000).

The decline in aggregate stability (described above) at the fifth month harvest may have been due to the macrocosms becoming root bound and the physical activity of the roots, eventually breaking down aggregates. However, this alone could not be the main reason, since the reduction in aggregate stability was also observed in the bare soil treatment. The decline in stability therefore, could be the result of the watering schedule over the course of the experiment. Macrocosms may have become too dry or wet over the course of the hotter summer months, causing more extreme wet-drying cycles which are known to influence aggregate formation and destruction (Lynch and Bragg, 1985; Amézketa, 1999; Denef *et al*., 2002). There is much debate over the effect wet-drying cycles can have on aggregates. Some authors (e.g. Dexter, 1988; Singer *et al.*, 1992; Oades 1993) have suggested that wet-dry cycles improve aggregate formation and stability whereas others (e.g. Tisdall, Cockroft and Uren, 1978; Mulla, Huyck and Reganold, 1992) found that wetting and drying cycles decreased macro-aggregation. More recent work by Denef *et al*. (2001) suggested that initial wet-dry cycles may improve aggregate formation/stability, without further effects after two wet-dry cycles. Furthermore the disruptive effect of re-wetting aggregates can be related to the speed of wetting. Kemper and Rosenau (1986) and Amézketa, Singer and

Le Bissonnais (1996) found that slow wetted aggregates maintain their structure, whereas fast wetting (e.g. during watering at the surface to prevent cracking, as undertaken here) has disruptive effects on aggregates (Cosentino, Chenu and Le Bissonnais, 2006). Therefore, the change in aggregate stability in this experiment could have been a result of fast wetting taking place within the macrocosm during fast watering events particularly at the soil surface.

4.4.3 EFFECT OF DEPTH ON SOIL STRUCTURE

As expected, total porosity decreased with depth within the macrocosm and was lowest at 135 mm from the top of the soil surface, where the weight of the overlying soil compacted this area. These data also correspond with mean pore size, pore perimeter, nearest neighbour distance and pore sphericity values, where larger pores (with larger pore perimeters and which were closer together and elongated) were found as one moved up through the soil macrocosm to areas under less pressure from surrounding soil (i.e. 35 mm from the soil surface). At this highest measured point within the macrocosm (35 mm from the soil surface), total porosity decreased (despite the mean pore size being the largest). Assumptions can be made that this may be due to surface watering slightly compacting the soil while trying to reduce the chance of cracking on the macrocosm soil surface.

4.4.4 *IMPACT OF DILUTION LEVEL AND TREATMENT ON SOIL STRUCTURE AND BIOLOGICAL PROPERTIES*

Organic matter content was highest within the macrocosms containing *P. lanceolata* and AMF, possibly due to roots and mycorrhizal fungi limiting the decomposition of organic matter (by sequestration), in addition to enhancing organic matter accumulation, e.g. by root production and turnover, and mycelial networks (Tisdall and Oades, 1982). The requirement for organic substrates within the soil macrocosms may be lower in soils amended with the 10^6 dilution in comparison to the 10^1, where microbial soil biomass was higher. This is supported by the greater proportion of Biolog C-substrates utilised by communities originating from the 10^1 dilution-treated soils. In general, organic matter content was correlated with aggregate stability and aggregate size distributions (measured by ASD_{CU}) to a greater extent than dilution level (and thus microbial diversity). Thus larger and more stable aggregates were observed in soils amended with the 10^6 dilution, where organic matter content was highest. This was particularly pronounced within the bare soil treatment at the 10^6 dilution where organic matter, ASD_{CU} and aggregate stability were all high. The impact of organic matter on aggregate stability followed a close relationship (Figure 4.53) that is widely acknowledged particularly from research by Tisdall and Oades, (1982); Chaney and Swift, (1984); Haynes and Beare, (1996) and Milne and Haynes (2004).

Within the bare soil at the 10^1 dilution, the rate of total substrate utilisation (Biolog data) was higher, but bacterial diversity was lower, than that of the 10^6 amended soil. This suggests one of several possible reasons; namely, there was a high degree of

metabolic (functional) redundancy within the bacterial community, that species present in the 10^6 amended soils were more 'Biolog active' than species from the other dilution treatment, or that increased C-utilisation reflected the C-source availability in the organic matter. Lower bacterial diversity resulted partly from a reduced abundance of TRFs 340, 369 and 405 bp from soils originating from the 10^1 dilution. Increased total porosity and larger mean pore sizes, pore perimeters and nearest neighbour distance were observed in the 10^1 diluted soils suggesting that in this case reduced diversity enhanced pore size, but limited pore occurrence. Moreover at the lower diversity, pore sphericity was reduced.

In the planted macrocosms with AMF, the opposite effects of dilution level on bacterial diversity were observed, with the macrocosms at the 10^1 dilution having a higher bacterial diversity (and greater abundances of fragments 340, 369 and 405 bp). Furthermore the rate of carbon substrate utilisation was lower than that observed in the the 10^6 amended soils. Despite the opposite effect of dilution level on bacterial diversity within the soil, similar effects of diversity on soil structure measurements were observed (compared to the bare soil) with mean pore size, pore perimeter and nearest neighbour distance all higher at a lower diversity (i.e. the 10^6 dilution) and sphericity highest within the more diverse soil (i.e. the 10^1 dilution).

In the planted macrocosms without AMF the influence of dilution level on soil microorganisms and soil structural measurements were more inconsistent, especially since no significant affect on diversity was observed between the two dilution levels. In

terms of the microbial measurements, soil biomass was highest in the 10^1 soil dilution than the 10^6 dilution, suggesting more living (or even dead microbes) were present within these macrocosms that may improve nuturient availability of vital nutrients that may otherwise limit plant and root development. In comparison to the bare soil treatment the relative abundance of fungal fragment length 75 bp was opposite with a higher abundance at the 10^6 than 10^1 dilution. In terms of soil structure measurements only nearest neighbour distance was influenced by dilution with, NND lower at the 10^1 dilution.

Generally therefore within this experimental system, a less diverse soil led to improvements in soil structure (in contrast to hypothesis two (section 1.5)). In this context soil structure may be defined as an 'ecosystem process'. Intuitively one may expect the opposite to occur, i.e. greater diversity results in improved soil structure. However, a study conducted by Wardle *et al.* (1997) on the influence of island area on ecosystem properties, concluded that on the small islands studied, plant species diversity was highest, but ecosystem process rates were lowest. These authors stated that in communities (islands) with the greatest diversity, dominance of species was prevented, but the species present had traits that retarded ecosystem processes. Certain parallels may be drawn with the current investigation although it should be remembered that only structural traits of soil are under consideration, i.e. just one functional role.

The effect of species diversity loss on functioning (such as soil structure development) will depend on the degree of loss the ecosystem undergoes. Chaplin *et al. (*2000) highlighted that extinction is a natural process, but when it occurs at an unnaturally rapid rate as is the case today, impacts on the functioning of ecosystems will occur. Furthermore Hunt and Wall (2002) investigated small scale losses of 15 functional groups, and found that soil still maintained itself despite a small decline in ecosystem service, provided that the loss of these species is compensated for by surviving groups. As microbial communities within soil are involved in numerous complex interactions, the soil should not be studied as a black box (Kennedy and Smith, 1995) and all communities and their processes need to be examined in relation to the entire ecosystem.

4.4.5 LINKING SOIL PHYSICAL PROPERTIES WITH SOIL MICROBIAL MEASUREMENTS

Within the soil macrocosms root activity appeared to have a dramatic impact. An initial relationship between root- and microbial-biomass highlighted the importance roots have on biomass-C, similar to that observed by Filho *et al. (*2004). As root biomass increases, the root surface area exposed to the soil increases, providing a larger area for exudates to be released, and these act as substrates for microbes within the soil, thus increasing biomass-C. The importance of roots within the soil environment on microorganisms has been highlighted by Lynch and Whipps (1990) and Bardgett (2005). Both root- and microbial-biomass had a negative impact on aggregate stability with more stable aggregates associated with lower levels of root- and microbial-biomass. This suggests that aggregate stability would increase when there is less

microbial activity with fewer competitive or synergistic interactions occurring, or with less demand on root exudates that improve the binding ability of a soil (Czarnes *et al*., 2000). Root biomass also influenced water repellency of aggregates with aggregates becoming more repellent with an increase in root material. This relationship would be as expected since roots are known to release polysaccharides and other exudates that act as binding agents to soil particles, but are known for their water repellency properties too (Czarnes *et al*., 2000; Hallett, Gordon and Bengough, 2003; Read *et al*., 2003).

Total porosity increased significantly with enhanced bacterial species richness (as proposed in hypothesis two in section 1.5). As bacterial numbers within the soil macrocosm increase, there would be an increase in exudates released, leading to increased binding of soil particles within the soil and thus increasing porosity within the soil environment. However, since the soil system is such a dynamic environment, the reverse could also be true with the soil porous network influencing microbial populations. As porosity increases within a soil environment, this would in turn suggest an increase in water, air and substrate flow throughout the environment, hence leading to an improvement in soil condition (i.e. those well aerated with improved substrate fluxes) which would be ideal for soil microbes (i.e. bacteria) and allow more species to survive in this environment. As seen in the biologically stained images from section 3.4.1, soil microbes tend to be located within regions of pore space, an observation in agreement with others (e.g. Foster, 1988), hence as these pore environments increase, the number of bacterial species inhabiting these areas would also increase. Despite

these assumptions no real conclusions can be made from these results as to whether pore size is an important property in controlling microbial population as stated in hypothesis 1 (section 1.5).

4.5 CONCLUSION / SUMMARY

- In the bare soil and planted AMF macrocosms the impact of dilution had differing effects on microbial communities. Nevertheless, within both treatments low bacterial diversity resulted in a larger mean pore size, pore perimeter and nearest neighbour distance, with pore sphericity decreasing. Such effects of diversity on soil structure were not observed in the planted non-AMF macrocosms. Therefore it appears the impact biodiversity losses have on soil structure are idiosyncratic since the impact of biodiversity loss depends on the soil conditions and structural features measured.

- As number of bacterial species (i.e. richness) increased, soil porosity increased; this however could also be observed in the reverse light, with porosity influencing bacterial richness.

- Mycorrhizal fungi inhibited root growth at the start of the investigation, but to a lesser degree at the later harvest periods. The presence of AM fungi within the macrocosm also increased aggregate stability, despite decreased aggregate and mean pore size. In contrast, total porosity and pore perimeter were highest within the planted non-AMF treatment, highlighting the dominance roots have

on soil structure. Furthermore, in the absence of plants, microbial biomass, carbon substrate utilisation and fungal species richness were lowest, but these parameters increased organic matter content within the soil.

- Generally it appeared that the physical and biochemical changes roots induce in the soil had the greatest impact on soil structural development within the soil macrocosms.

- Aggregate stability was controlled by the organic matter content within the macrocosms that was generated by the presence of roots and AMF within the soil ecosystem.

- Soil structure development took place within all macrocosm (regardless of treatment) after an initial settling period where the soil compacted due to gravity, with increases in porosity of up to 59 % after 4 months, illustrating that soil microbes, roots and AMF are vital in developing, stabilising and maintaining a soil's function.

5 EXPERIMENT 3: IMPACT OF MYCORRHIZAL FUNGI ON SOIL STRUCTURE DEVELOPMENT

5.1 INTRODUCTION

It is widely acknowledged that arbuscular mycorrhizal fungi (AMF) are a major factor which influence aggregate formation and pore size distribution within soil (section 1.3.3). Although there has been an increasing quantity of research taking place on AMF and on soil structure in particular, there appears to be little focus on the mechanisms and how their interactions lead to the observed changes in soil structure. Andrade *et al. (*1998) investigated the relationship between AMF, groups of rhizobacteria and soil aggregation, and described the changes through the activity of AMF, but not the reasons behind these changes. Andrade *et al. (*1998) concluded that mycorrhizal fungi increased the number of water stable aggregates and that indirectly influenced the microbial populations, through the alteration and creation of pore spaces. In addition, Bearden (2001) also suggested mycorrhizal activity within soil caused significant changes in pore size and distribution; however how these changes took place was not investigated. The recent literature has focused on assessing the effect AMF have within the soil environment. However, it is vital that exploration now focuses more on the role mycorrhizas have on soil aggregation and in particular isolation of individual factors that are involved in aggregate formation and stability. As Rillig and Mummey (2006) reported, given the importance of mycorrhizal fungi to the functioning of the soil ecosystem, relatively little work has been focused to this area. From a search in Web of Science, only ~ 8 % of all articles dealing with mycorrhizal fungi had soil structure in

the title, abstract or key words, whereas only ~ 0.9 % had aggregate, thus illustrating how little work has been dedicated to this area.

Studies have been undertaken to determine the effect that different mycorrhizal fungi have on plant and root systems. Generally, different species of AMF lead to different colonisation rates within the same plant species (Oliveira *et al.,* 2006). Furthermore, mycorrhizal fungal diversity has been found to influence plant productivity and community composition (van der Heijden *et al.,* 1998a, Harnett and Wilson, 1999). It has been acknowledged that plant species diversity decreases with the presence of mycorrhizal fungi (Harnett and Wilson, 1999; Klironomos *et al*., 2000) despite increased plant productivity (Klironomos *et al*., 2000). The reverse of these findings have also been discovered with plant community composition influenced by the presence of differing mycorrhizal diversities (Johnson *et al*., 2003). However, Oliveira *et al. (*2006) suggests the species of AMF within a root system will determine how plant species coexist and the degree of plant growth according to the mycorrhizal species present.

Different AMF species, within the rhizosphere, have also caused differing affects on plant biomass with individual plant species (van der Heijden *et al.,* 1998b; Oliveira *et al.,* 2006), in addition to changes in root morphology (van der Heijden *et al.,* 1998b; Copetta, Lingua and Berta, 2006), phosphorous concentrations in leaves (Oliveira *et al*., 2006) and seed spikes (Oliveira *et al*., 2006). Different AMF species within root systems have also been observed to have different amounts of extraradical mycelium (ERM), that can have different efficiencies in the uptake of phosphate from soil (Jakobsen, Abbott and Robson, 1992; Helgason *et al.*, 2002; Munkvold *et al*., 2004;

Jansa, Mozafar and Frossard, 2005; Avio *et al*., 2006 and Oliveira *et al*., 2006) that could ultimately contribute to differential growth of plants. Furthermore, van der Heijden *et al. (*1998b) also found that as the number of AMF taxa within the plant root increased, the quantity of shoot and root biomass increased until a certain point where biomass started to decline. These authors therefore suggested that with a loss of AMF diversity, plant biodiversity and ecosystem productivity decreases while the ecosystem becomes unstable. Such results indicated that AMF diversity does have an effect on plant and root biomass, ERM and seed production but what has not been studied in any great detail is the effect AMF diversity has on soil structure. Fitter (2005) suggested soil aggregation to roots required ERM that were located closer to the roots, hence species like *G. geosporum* which produce small amounts of ERM (Green *et al*., 1994; Oliveira *et al.*, 2006) may influence soil structure more readily (particularly near the root) than species which have larger ERM (normally associated with phosphate acquisition in phosphate low soils (Bago *et al*., 2004)) such as *Glomus fasciculatum* (Abbott, Robson and de Boer, 1984). This suggests the effect of AMF species on soil structure within the rhizosphere may be influenced by external soil properties such as the phosphorus content and hence highlights the dynamic nature of this environment. Moreover Piotrowski *et al*. (2004) found that the presence of both *Glomus intraradices* and *G. etunicatium* within *Plantago lanceolata* roots caused increases in water stable aggregates (WSA) within the soil, however these effects were found to be significantly lower than that of other mycorrhizal species. This therefore suggests that the effect of AMF on aggregate stability is dependent on mycorrhizal species.

The aim of this investigation was to determine the effect on soil structure, as measured by image analysis and aggregate stability, of differing levels of microbial diversity, in soil macrocosms containing *P. lanceolata* and differing mycorrhizal fungi species. Although roots and AM fungi have been shown to influence soil structure through their activity, the relative importance of this has not, to date, been properly assessed. Through the use of split column systems within this investigation, the importance of both hyphae and root material on soil structure was assessed. Furthermore the effect of mycorrhizal fungi species on the development and maintenance of soil structure was monitored, in relation to the effect differing background biodiversity levels have on the soil structural development. This investigation therefore, aims to provide some evidence of the effect AMF colonisation of *P. lanceolata* has on soil structure development, by separating hyphal activity from that of roots using split macrocosms.

Soil structural development was examined by using X-ray CT scanning, combined with traditional techniques such as aggregate stability and aggregate size distribution. Microbial analysis was undertaken using the novel technique of T-RFLP in addition to microbial biomass measurements. Experimental macrocosms were inoculated using the dilution technique (Griffiths *et al.*, 2001) to give two levels (+/-) of indigenous microorganisms. These 'background' levels of microorganisms, together with addition (or not) of arbuscular mycorrhizal fungi (AMF) created an experimental regime in which the effects of soil biodiversity on soil structure development could be determined.

The aim of this chapter is to test hypothesis two, three and four (section 1.5).

5.2 MATERIALS AND METHODS

5.2.1 MACROCOSM CONSTRUCTION

Air dried sterile Dunnington Heath (sandy loam) was packed into split plastic columns (19.7 cm length x 10.8 cm internal diameter) to a bulk density of 1 g cm^{-3}. Mesh of 20 µm (Cadisch Precision Meshes Ltd, London, UK) was glued to the edges of one half of the column and pulled tight across the column to act as a barrier through the middle of the column (Figure 5.1). This mesh was trimmed flush to the top of the column and cut with an additional 2.5 cm from the base of the column, which was glued to the base mesh to prevent contamination across the column sides. In addition, 400 µm mesh (Cadisch Precision Meshes Ltd, London, UK) was glued to the column base to allow capillary rise of water from base trays to prevent cross contamination of soil columns through watering. These columns were secured together using Duck® tape. These split columns allowed separation of the effects that roots plus hyphae (rhizosphere) have on soil structure in comparison to just hyphae (hydrosphere).

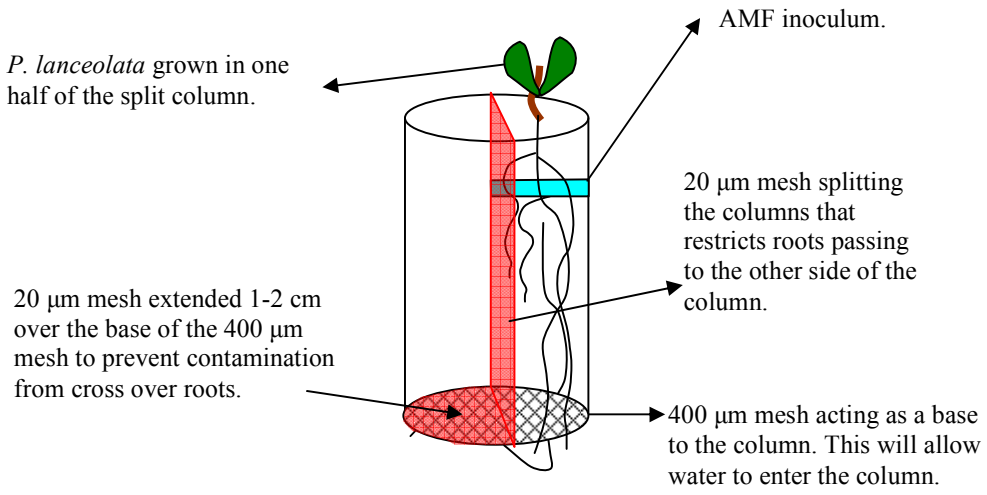


Figure 5.1: Split macrocosm design.

The constructed macrocosms (Figure 5.1) were packed loosely and uniformly with soil, by pouring equal quantities of sterile soil into each side of the mesh. At a depth of 14 cm (from the base of the column) a layer of AM fungal inoculum (PlantWorks Ltd, Sittingbourne, Kent, UK) was placed into one side of the column. The AMF inoculum used in this investigation consisted of three different AMF species: *Glomus geosporum* (species A), *G. mosseae* (species B) and *G. intraradices* (species C). Inoculum was allocated to one half of the split macrocosms individually and in every combination of the species to give the following species groupings, A, B, C, AB, AC, BC and ABC. Non-mycorrhizal treatments were given sterilised inoculum. A total of 12 g of inoculum was used within each half of the macrocosm where the seeds were to be later sown. This was split appropriately according to treatment: The control received 12 g sterilised inoculum; the one species inoculum consisted of 6 g of the appropriate species and 6 g of sterilised inoculum; the two species mix consisted of 3 g of each species and 6 g of sterilised inoculum; the three species mix consisted of 2 g of each individual AMF inoculum and 6 g of sterilised inoculum.

5.2.2 BACKGROUND MICROBIAL INOCULATION

Half of the soil macrocosms were inoculated with indigenous microbes using a soil slurry solution, made from field fresh soil by diluting it in sterile ¼ strength Ringers solution. Fresh soil was taken from the field site (section 2.1.2) on the day it was required. The soil slurry solution was made to a dilution of 10^{1}. At inoculation half the soil macrocosms were left to saturated in the 10^{1} soil slurry solution and the other half saturated in sterilised ¼ strength Ringers solution overnight to create two different

microbial diversities (Salonius, 1981; Griffiths *et al.,* 2001). Once the cores were saturated, they were removed from the solution and left to drain for 2 days to reach field capacity and weighed. At the start of the experiment, three unsterilised seeds of *P. lanceolata* were sown into the top of the macrocosms. Once seedlings had reached the one true leaf stage of growth, seedlings were thinned to leave one seedling remaining in the split macrocosm (Figure 5.2).

Macrocosms were maintained in a glasshouse at 20-30 ^{o}C with a 16 hour daylength supplemented by lights. Soil macrocosms were maintained at field capacity (determined by weight) by watering with sterile (autoclaved at 121 ^{o}C and 15 PSI) deionised water.

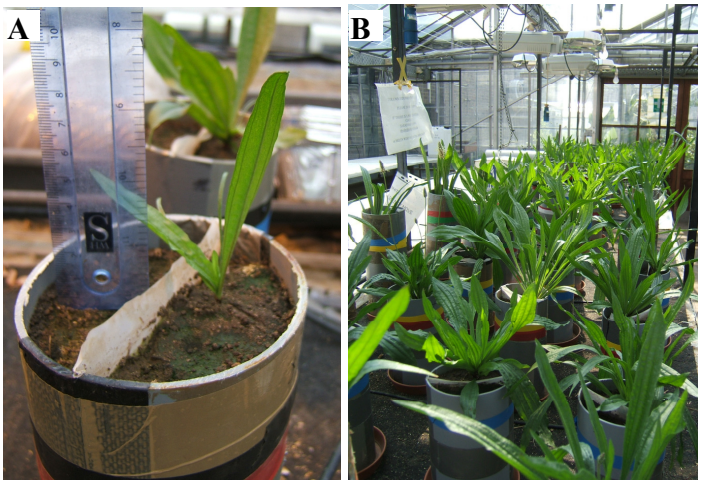


Figure 5.2: Example of A) split soil column with *P. lanceolata* and B) glasshouse containing the macrocosms.

5.2.3 TREATMENTS AND REPLICATION

The soil macrocosms were allocated to one of sixteen different treatments; all macrocosms were sown with *P. lanceolata* and were amended with either a 10^{1} dilution of soil slurry or sterile ¼ strength Ringers solution. In addition, the columns contained one of the following: (1) Sterilised AMF inoculum, (2) *G. geosporum* (3) G. *mosseae* (4) *G. intraradices*, (5) *G. geosporum* plus *G. mosseae* (6) *G. geosporum* plus *G. intraradices*, (7) *G. mosseae* plus *G. intraradices*, (8) *G. geosporum*, *G. mosseae* plus *G intraradices*. Three replicate columns per treatment were destructively harvested after 7 weeks to assess plant biomass, mycorrhizal colonisation, soil microbial communities and soil structure. An additional (fourth) replicate from each treatment was scanned using X-ray CT at the beginning and end of the experiment to allow changes in porosity and mean pore size to be accessed over time. For logistical reasons these columns were not destructively harvested. A total of 48 columns were harvested during the experiment.

5.2.4 HARVEST REGIME AND MEASUREMENTS

Soil macrocosms were destructively harvested seven weeks after germination and microbial and structural assessments carried out as described in sections 2.1.3, 2.2 and 2.3. Additional measurements specific to this experiment are described below.

5.2.5 HYPHAL PENETRATION

In order to determine whether AM hyphae had penetrated the dividing mesh, a modification of the method described by Jakobsen, Abbott and Robson (1992) was used.

5.2.5.1 Experimental procedure

Two grams of soil were added to 250 ml of de-ionised water and shaken vigorously by hand. Samples from the **planted** size of macrocosms (containing root material) were poured through a 710 μm sieve into a Waring Blender before blending at high speed, whereas samples from the **unplanted** side of the macrocosms were directly poured into the Waring Blender before blending at high speed. The blended solution was transferred into an Erlenmeyer flask before adding a 10 ml aliquot of 0.1 % lactoglycerol-typhan blue solution (1:1:1 v:v:v ratio of 80 % lactic acid, glycerol and de-ionised water to typhan blue powder). The solution was agitated vigorously at high speed before leaving for 10 minutes to stain and settle. Triplicate 7.5 ml aliquots were pipette onto 25 mm Millipore glass fibre filters (1.2 μm pore size (Fisher Scientific, Leicestershire, UK)) in a filtration manifold holding. Once filtered the Millipore filters were transferred to microscope slides and viewed. Presence of blue stained hyphae across each Millipore filter was determined by scoring each Millipore filter by the presence or absence of hyphae within a field of view at 10X magnification, to calculate percentage occurrence.

5.2.6 X-RAY CT ASSESSMENT

Soil columns were scanned non-destructively using a Venlo H series, high resolution X-ray CT Scanner (H 350/225 CT; X-TEK, Tring, Hertfordshire, UK). Each column was scanned at 296 kV and 2.3 mA with an exposure time of 220 mS. Prior to scanning, the sample was placed onto a movable sample stand, 145 mm away from the detector (Figure 2.5). A 2 mm primary copper filter was placed near the X-ray source to eliminate X-ray scatter in addition to a 4 mm secondary copper filter placed at the detector to prevent detector saturation (i.e. when the input to the detector exceeds the total capacity) and beam hardening. Soil columns were scanned at various depths throughout the macrocosms to allow assessment of the soil pore system at various depths. Macrocosms were scanned at a depth of 60, 80, 100, 120, 140 and 160 mm from the top of the soil surface. A marker was placed to one side of the macorcoms to identify the planted side of the split macrocosm in X-ray CT images.

5.2.7 IMAGE ANALYSIS

Images obtained using the CT scanner were initially processed in Image J®, to allow image rotation prior to further image processing and assessment using AnalySIS® (Soft Imaging Systems (SIS), Münster, Germany) to isolate pore spaces. The image resolution was 64 μm pixel $^{-1}$. Since split columns were used in the experiment, the initial image was cropped either side of the central mesh to a box size of 2688 by 7168 μm. This allowed assessment on the soil structure at both sides of the split column and removed boundary effects on the structural assessment (Figure 5.3).

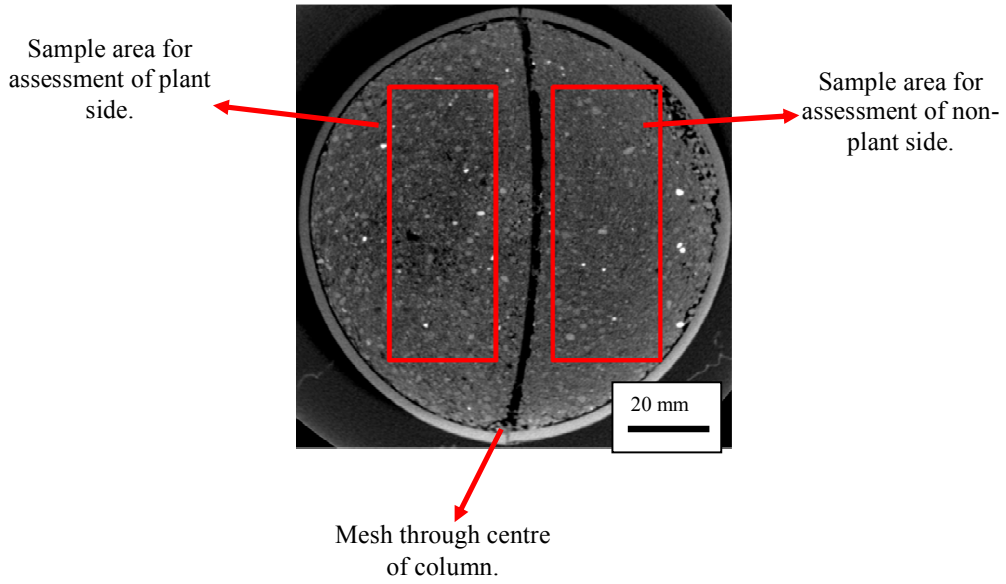


Figure 5.3: Selection of image area from split macrocosms for image analysis.
Note: Black indicates pore space, in addition to the black line within the centre of the column indicating mesh.

Filtering was performed using: 1) A Median filter which smoothed the image; 2) a SharpenII filter, that emphasised detail; 3) a Lowpass filter, that acted as a noise reduction filter to reduce the influence of artefacts; 4) image conversion to greyscale. Binarisation of the images was undertaken using a modified auto-threshold (where the overflow value was set as 32.7 %), since default settings did not produce satisfactory results separating solid from the pore phase. Finally an erosion morphological filter was applied with a hexagon lattice setting of size three pixels and two iterations (Figure 5.4).

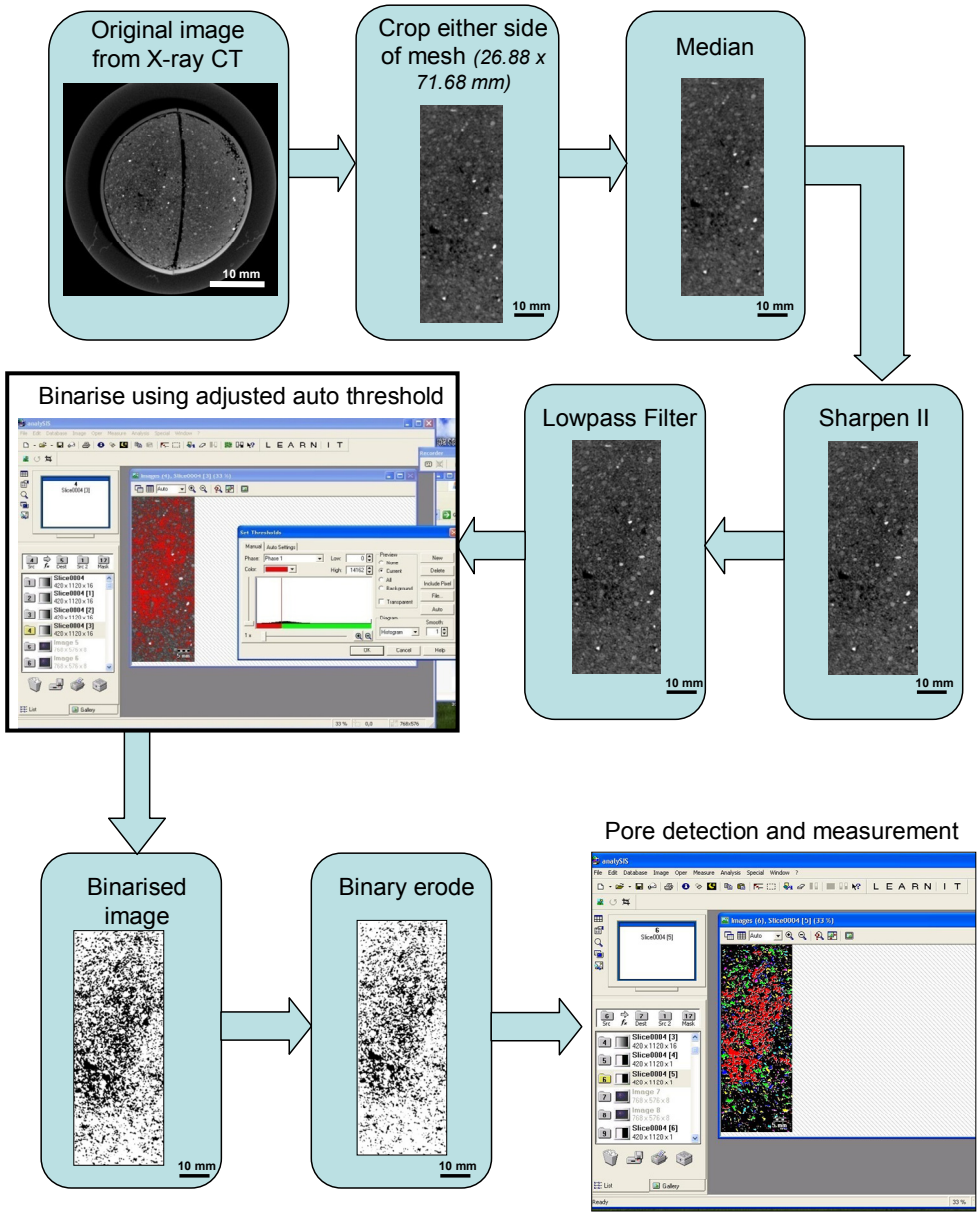


Figure 5.4: Image analysis sequence using Analysis®.
NB: Pore space is indicated in black within the image.

During the post experimental image assessment, it was noticed that ring artefacts had an important influence on pore size and shape within these X-ray CT scans (Figure 5.5). To reduce the influence of ring artefacts, a polar transformation was applied to the

image (prior to the image sequence stated above) using polar co-ordination. After transformations, three convolve filters were applied before the image was reverted back to cartesian co-ordination. Since polar transformation is useful to unwrap images of rounded objects, it is an ideal method to remove ring artefacts prior to 'bending' of the image using the Cartesian transformation. For most images polar transformations removed all evidence of ring artefacts, however within some images these artefacts were too great to completely remove.

Morphological analysis was performed on all images using AnalySIS®, this included the following pore measurements; porosity (total percentage pore area of the sample); mean pore area (average pore size of the pores within the sample); sphericity (described as the circularity of pores calculated by central moments (while also being a function of pore size), with sphericity increasing with smaller pore size, defined as 1 = circular and 0 = elongated and flat) and nearest neighbour distance (an assessment of the average distance between pores from centre to centre, providing a sign of structural development).

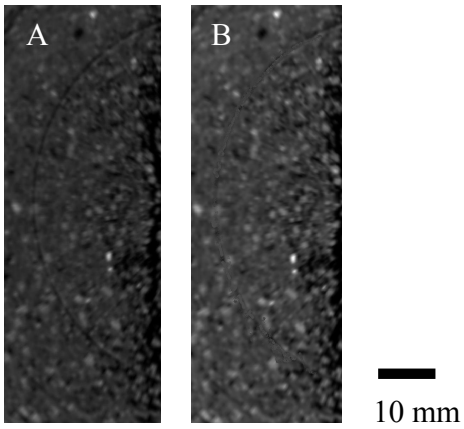


Figure 5.5: Example of image with a) a ring artefact taken directly after CT scanning and b) after image transformation using a polar transformation and convolve function in Image J.

5.2.8 *STATISTICAL ANALYSIS*

Data were subject to analysis of variance (ANOVA) using Genstat version 11.1. Factors included supplemented indigenous microbes, AMF species mix and location within the split macrocosm (i.e. planted or unplanted side of the central barrier). Data for percentage root length colonised by mycorrhizal hyphae, arbuscules and vesicles in addition to hyphal penetration data were arcsin square root transformed. Mean pore area and sphericity measurements from image analysis were transformed using $\log_{10}+2$ and $\log_{10}+1$ respectively to normalise the data and satisfy the requirements of ANOVA. For T-RFLP data PCA analysis was undertaken using Genstat 11.1 and Minitab 15.

5.3 RESULTS

5.3.1 *ARBUSCULAR MYCORRHIZAL COLONISATION*

Percentage root length colonised by mycorrhizal fungi ranged from 5-40 % depending on the species combinations. No colonisation was observed in the non-mycorrhizal treatments. The greatest percentage colonisation was observed in treatments containing *G. geosporum* (singly or in combination) ($F_{7,32} = 7.66$, $P < 0.001$). Addition of background indigenous microbes (soil slurry amendment) resulted in reduced AMF colonisation relative to that of the non-amended treatments, but only when AMF were present in combinations of species (Figure 5.6). When cultured as single species, slurry amendment had no effect on percentage root length colonised (AMF x slurry amendment interaction, $F_{7,47} = 2.58$, $P < 0.032$).

Arbuscules were observed in most of the mycorrhizal treatments, but to a lesser degree than hyphal colonisation (Figure 5.6). Soil slurry amendment reduced the number of arbuscules present, in tandem with reducing hyphal colonisation.

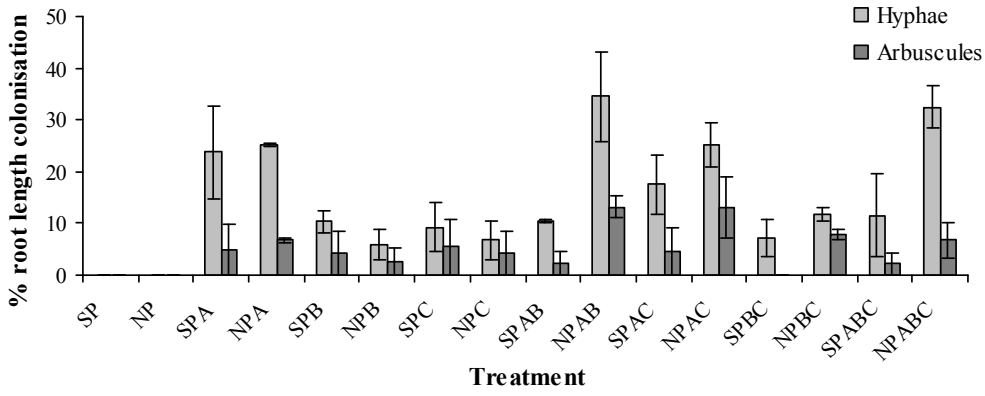


Figure 5.6: Mycorrhizal colonisation of roots by hyphae and arbuscules.
Data are means (arcsin square root transformed) ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

5.3.2 SHOOT AND ROOT BIOMASS

Neither addition of soil slurry (indigenous microbes) nor AMF inoculation significantly affected shoot dry weight, although there was a slight trend towards increased biomass in the treatments containing *G. mosseae* and *G. intraradices* in combination (Figure 5.7). A similar trend was significant ($F_{7,30} = 4.57$, $P = 0.001$) for root biomass when the two AMF species were combined and when the soil had been amended with indigenous microbes (Figure 5.8). The most interesting observation for both root and shoot biomass was that in the presence of indigenous microbes, any increase in biomass resulting from the 3-AMF species combination was lost when soil was amended with

slurry. Overall there appeared to be some benefit to the plant when at least two AMF species were present (Figure 5.9).

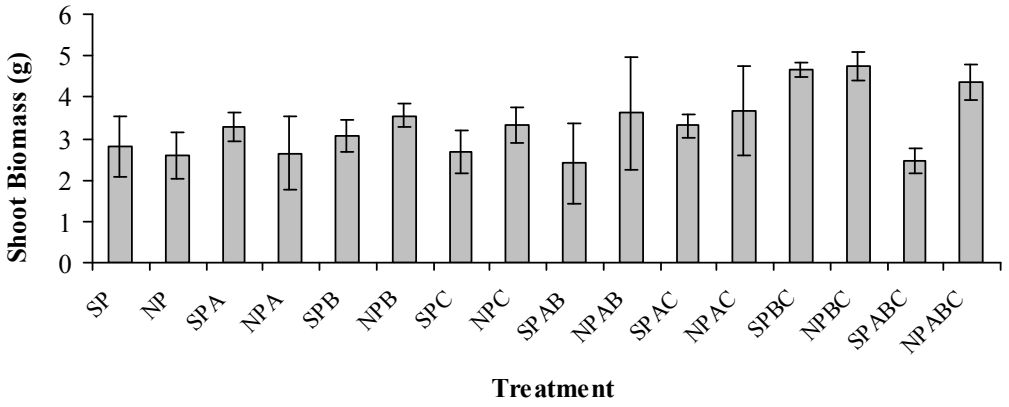


Figure 5.7: Dry shoot matter from each fungal treatment.
Data are means ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

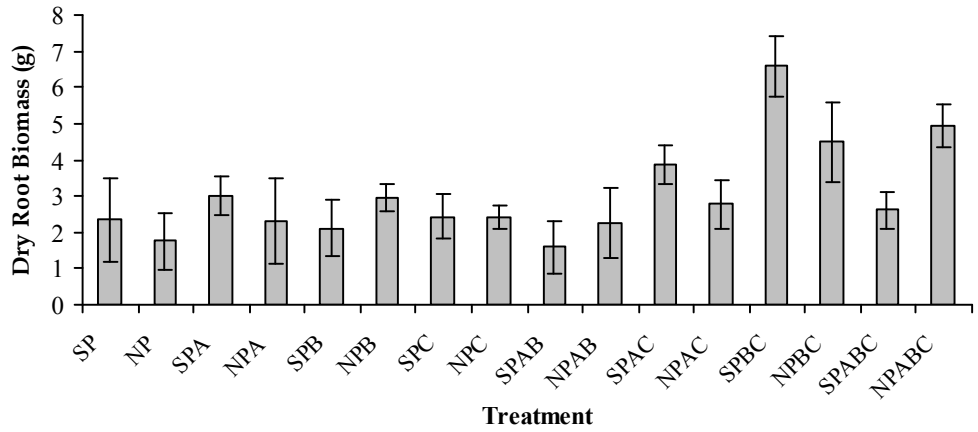


Figure 5.8: Dry root matter from each fungal treatment.
Data are means ± standard error. Treatment codes are as in Fig. 5.7.

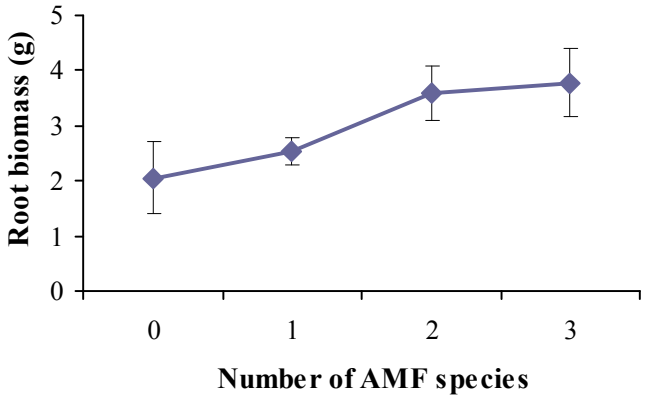


Figure 5.9: Effect of number of AMF species on root biomass within all treatments.
Data are means ± standard errors.

5.3.3 MYCORRHIZAL DEPENDENCY

When biomass is expressed as mycorrhizal dependency, the negative effect of adding soil slurry is highlighted in certain AMF species combinations, although this trend was not significant (Figure 5.10). Generally however, *P. lanceolata* benefited from inoculation with mycorrhizal fungi.

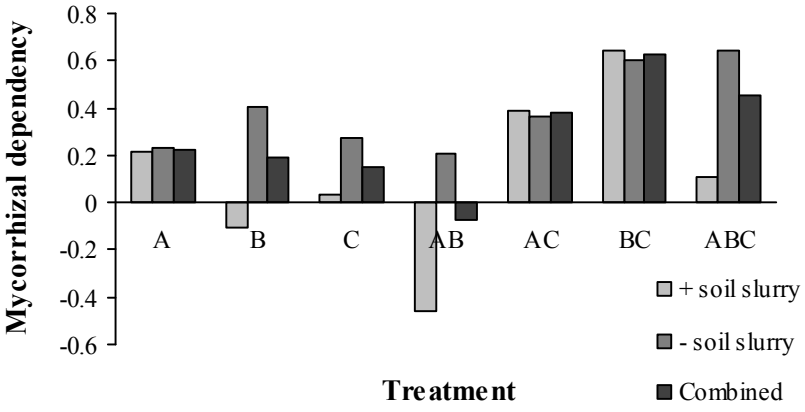


Figure 5.10: Mycorrhizal dependency of *P. lanceolata* with inoculation of *G. geosporum, G. mosseae and G. intraradices*.
(Treatment codes: Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

5.3.4 *HYPHAL PRESENCE IN BULK SOIL*

Hyphal presence is expressed as percentage occurrence within the soil sample. Location within the macrocosm influenced hyphal presence in the bulk soil, with more hyphae located in the planted side (14.6 %) compared to the unplanted side (12.9 %) ($F_{1,62}$ = 4.56, P < 0.037). Furthermore, fungal species mix also influenced hyphal occurrence, with all AMF mixtures resulting in a greater hyphal presence than that observed in non-mycorrhizal treatments (AMF as a single factor, $F_{7,62}$ = 11.90, P < 0.001). Hyphal presence was highest within the 3-species mix (16.3 %) (Figure 5.11).

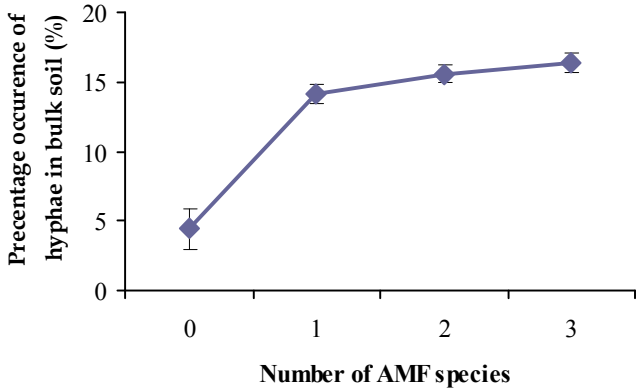


Figure 5.11: Effect of the number of AMF species within both sides of the macrocosms on hyphal presence within the bulk soil within the macrocosms ($F_{3,90}$ = 24.91, P < 0.001).
Data are means (arcsin square root transformed) ± standard error.

Within the sterilised AMF treatment, hyphal occurrences were highest in the amended soil (7.7 %) compared to the unamended macrocosms (1.1 %) suggesting that amendment with the 10^{1} dilution introduced hyphae into the soil (slurry x AMF interaction, $F_{7,95}$ = 2.27, P = 0.04) (Figure 5.12). It was not possible to determine whether the hyphae introduced with the soil slurry were saprophytic or mycorrhizal, but most likely the former. Nevertheless, all mycorrhizal additions (with the possible exception of *G. mosseae* as a single species inoculum) resulted in increased hyphal

observations relative to those of the slurry-only treatment on the unplanted side, indicating that AMF hyphae penetrated the central mesh barrier and accessed the unplanted side of the column.

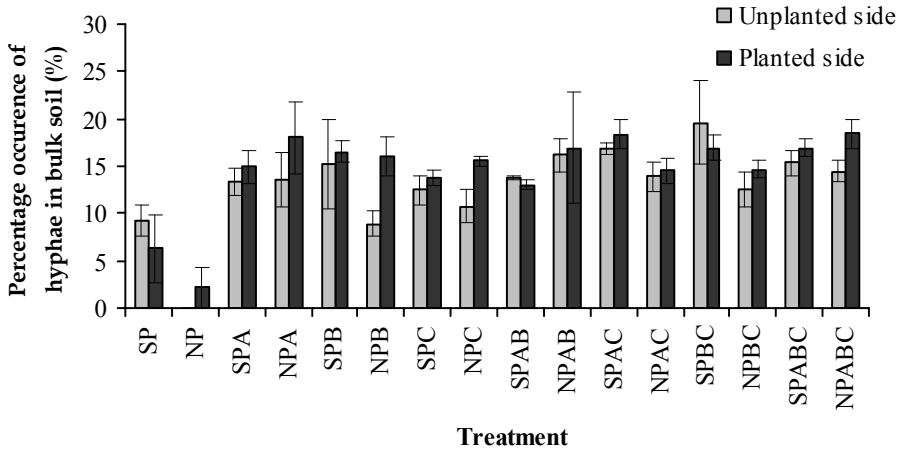


Figure 5.12: Percentage occurrence of hyphae within the bulk soil within the macrocosm either side of the mesh.

Data are means (arcsin transformed) ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*, B* – G. mosseae *and C* = G. intraradices).

5.3.5 ORGANIC MATTER

No significant differences in soil organic matter (OM) were observed in any of the treatments. The overall average percentage OM was 3.83 %.

5.3.6 MICROBIAL BIOMASS

Fungal species mix influenced microbial biomass-C within the split macrocosms. Soil within the macrocosms inoculated with *G. geosporum* plus *G. intraradices* had the highest microbial biomass of all fungal mixes (183 μg C g^{-1} soil). This was significantly higher than in those macrocosms containing all three AMF species (108.5

µg C g^{-1} soil) or *G. intraradices* (78.3 µg C g^{-1}) individually. A relatively high soil biomass was associated with *G. geosporum* individually (136.7 µg C g^{-1}) (species mix, $F_{7,54}$ = 3.75, P = 0.002; both side of macrocosms combined).

Microbial biomass was also significantly influenced by location within the macrocosm ($F_{1,54}$ = 10.18, P = 0.002), with soil from the planted side having a higher microbial biomass (134.3 µg C g^{-1}) than soil from the unplanted side (95.5 µg C g^{-1}).

Soil slurry amendment did not affect biomass-C in the absence of AMF on the planted sides of the columns, but did increase biomass-C within the unplanted sides of non-mycorrhizal columns. Slurry amendment had little effect on biomass-C in planted sides when *P. lanceolata* was mycorrhizal, with the notable exception of the *G. geosporum* inoculated treatment in which slurry markedly enhanced biomass-C (slurry x AMF interaction $F_{7,54}$ = 2.84, P = 0.014; Figure 5.13).

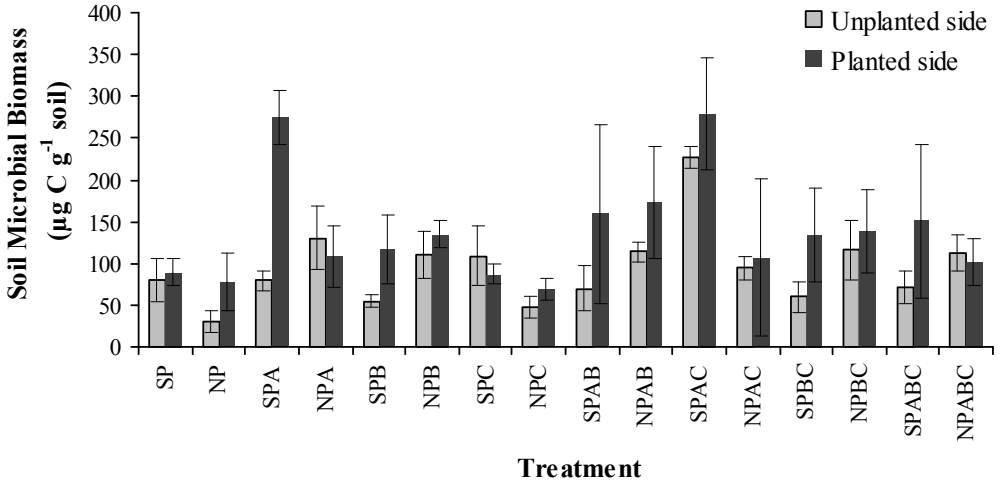


Figure 5.13: Microbial biomass C of the soil within the macrocosm either side of the mesh.

Data are means ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

5.3.7 TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM

Terminal - Restriction Fragment Length Polymorphism (T-RFLP) was carried out on DNA extracted from soil microbes under each treatment at the end of the experiment. As mentioned in section 2.3.3.4 relative abundance of each peak occurring at a dye signal greater than 100 units was included, with any shoulder peaks (associated with base pair addition through the use of PCR amplification) removed from analysis. Principal component analysis (PCA) using the covariance matrix was carried out on all T-RFLP fragment profiles by using relative abundance results. PCA was performed on bacterial and fungal datasets separately and then by location within the macrocosm.

5.3.7.1 Species richness based on T-RFLP

Microbial species richness was determined from T-RFLP (section 2.3.3). Neither soil slurry amendment nor AMF inoculum affected bacterial species richness. More bacterial TRFs were recorded from the planted side of the columns than from the unplanted (9 versus 7; $F_{1,61} = 8.26$, $P = 0.006$).

Adding soil slurry decreased fungal species richness in the absence of AMF inoculum on the planted and unplanted sides of the columns, with the unplanted sides containing more species than the planted sides. Slurry amendment increased species richness on the unplanted side of the column in the presence of *G. mosseae* as a single species, but had little effect on species richness in the planted side. In contrast, slurry amendment increased fungal species richness on the planted side of columns containing *G.*

geosporum plus *G. mosseae* and *G. geosporum* plus *G. intraradices* (Figure 5.14; AMF x slurry x location interaction, $F_{7,58} = 2.99$, $P = 0.009$).

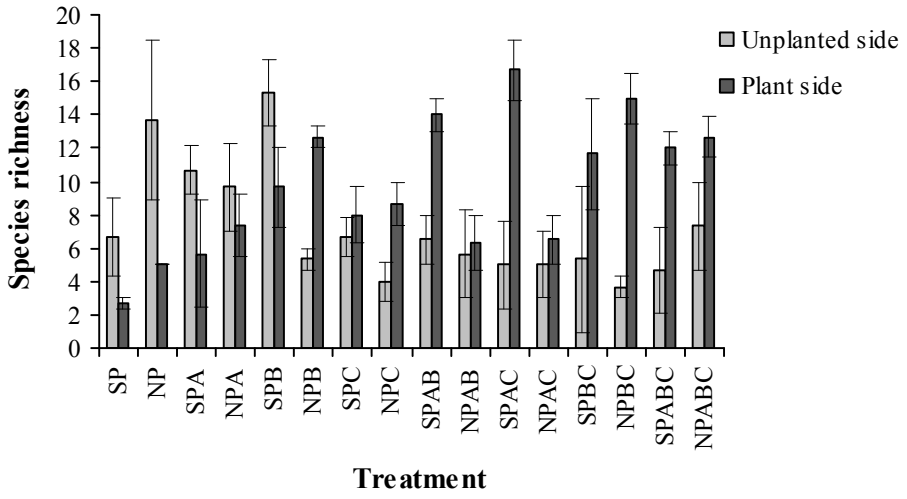


Figure 5.14: Fungal species richness for each side of the macrocosm for each treatment.
Species richness refers to the number of TRFs. *Data are means ± standard error.* *(Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment Codes containing A, B and C relate to AMF species with A* = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

Fungal species diversity decreased to a minimum when two AMF species were present in the inoculum in the unplanted macrocosms, whereas in the planted side species diversity increased with AMF species number (Figure 5.15).

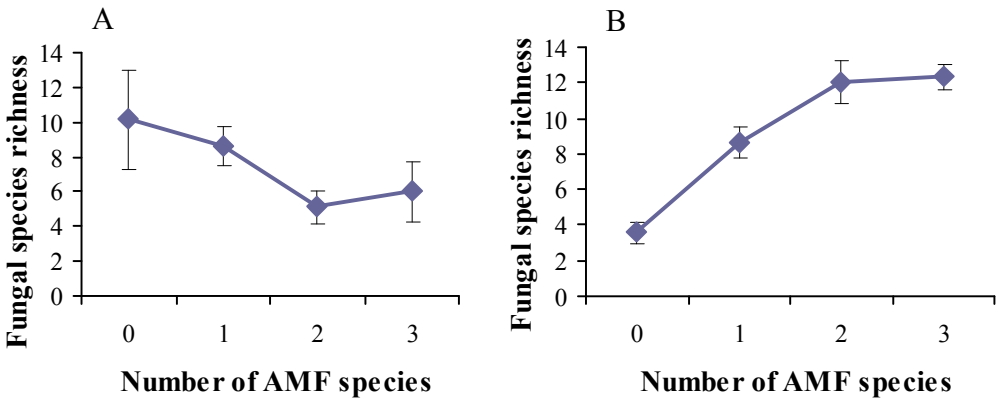


Figure 5.15: Effect of the number of AMF species within the macrocosms on fungal species richness on A) the unplanted ($F_{3,38}$ = 2.85, P = 0.05) and B) the planted side of the macrocosm ($F_{3,38}$ = 7.97, P < 0.001)).
Species richness refers to the number of TRFs. *Data are means ± standard error.*

5.3.7.2 Species diversity based on T-RFLP

TRF number and corresponding relative abundance data were used to calculate Simpson's diversity index. The greatest contrast in fungal species diversity resulting from slurry amendment relative to unamended treatments was observed in columns containing *G. geosporum* plus *G. mosseae* and *G. geosporum* plus *G. intraradices*. Here, amending with slurry increased soil fungal diversity. In the non-mycorrhizal columns and those containing *G. geosporum* as a single species, slurry amendment slightly reduced fungal biodiversity (slurry amendment x AMF interaction, $F_{7,55}$ = 2.53, P = 0.025; Table 5.1).

Bacterial species diversity increased to a maximum when two AMF species were present in the inoculum, but declined when all three AMF species were present (Figure 5.16).

Table 5.1: Simpson's fungal diversity index for whole macrocosms under each fungal mix and with the impact of slurry addition.

Fungal mix	Slurry amended soil	Unamended soil
Sterile	0.62 ± 0.060	0.78 ± 0.054
A	0.67 ± 0.098	0.76 ±c0.046
B	0.86 ± 0.019	0.75 ± 0.070
C	0.74 ± 0.053	0.69 ± 0.052
AB	0.76 ± 0.076	0.57 ± 0.12
AC	0.87 ± 0.023	0.59 ± 0.07
BC	0.72 ± 0.12	0.78 ± 0.045
ABC	0.83 ± 0.021	0.75 ± 0.081

Data are means ± standard error. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices.

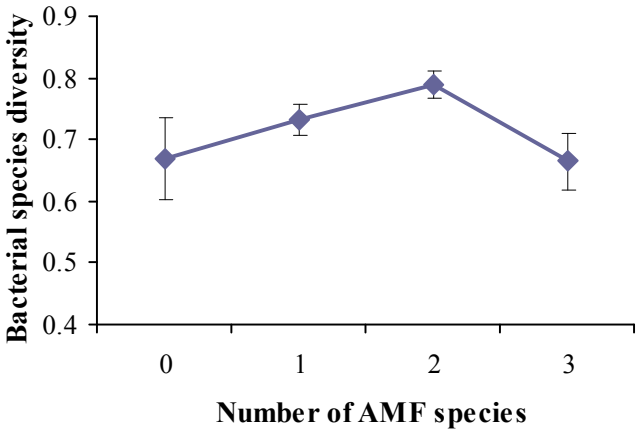


Figure 5.16: Effect of number of AMF species on bacterial species diversity within the planted side of the macrocosm.
Data are means ± standard error. ANOVA of data shows a significant difference ($F_{3,38}$ = 3.15, P = 0.036).

Location as a single factor was weakly significant for bacterial species, with greater diversity observed in the planted than the unplanted side of the macrocosm ($F_{1,55}$ = 3.78, P = 0.057). Fungal diversity was unaffected by location.

5.3.7.3 Relative abundance determined from T-RFLP

Through analysis of the relative abundance of common fragment lengths within the sample, it is clear that fungal communities were influenced by AMF species mix. Fragment 260 bp was only detected when *G. geosporum* was present individually or when mixed ($F_{7,55}$ = 17.63, P < 0.001; Figure 5.17), suggesting that this T-RF was actually *G. geosporum* that was present within the macrocosm soil. Fragment length 83 bp was also influenced by the presence of *G. geosporum* (individually and mixed) ($F_{7,55}$ = 2.81, P = 0.014), whereas fragment 326 bp was dominant in the non-AMF treatment ($F_{7,55}$ = 9.97, P < 0.001).

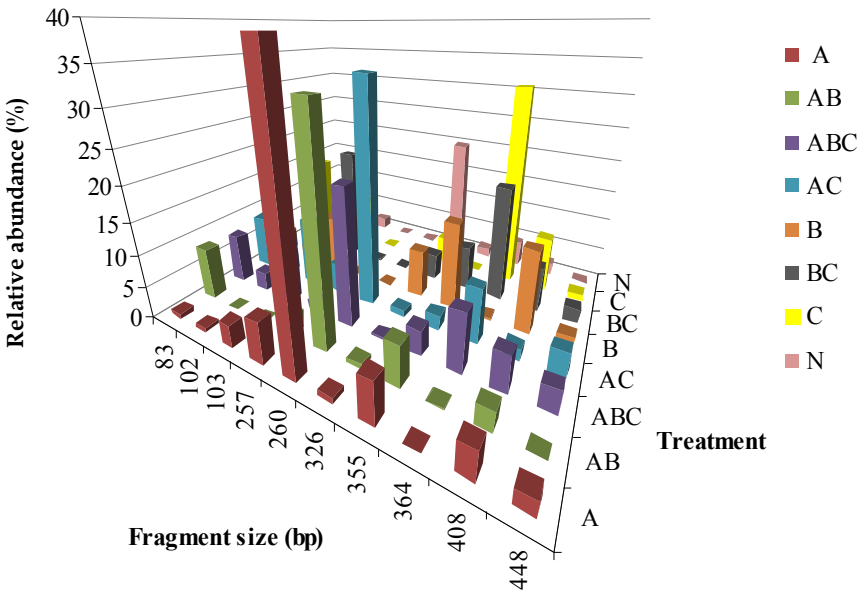


Figure 5.17: Relative abundance of most common fungal fragments from T-RFLP profiles.
No standard errors are shown for clarity. (Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

Furthermore, bacterial fragments present were influenced by AMF species mix, with fragment 371 bp occurring least in the macrocosms containing *G. intraradices*

(F_{761} = 3.66, P = 0.002). Generally however, bacterial fragment 372 bp appeared to dominate the profile (Figure 5.18). This fragment was influenced by slurry amendment ($F_{1,61}$ = 9.27, P = 0.003) and fungal mix ($F_{7,61}$ = 3.66, P = 0.002) as single factors, with the fragment more likely to be present in a non-amended soil and one which had either no addition of AMF or in the *G. mosseae* plus *G. intraradices* mix. Further effects of treatment and dilution were observed through PCA analysis as described below.

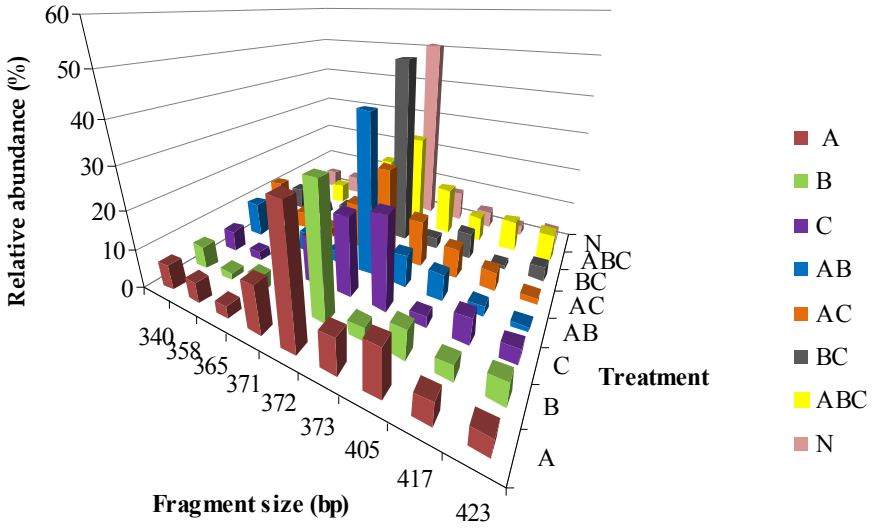


Figure 5.18: Relative abundance of most common bacterial fragments from T-RFLP profiles.
No standard errors are shown for clarity. (Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

5.3.7.4 Fungal T-RFLP

Principal component 1 (PC1) accounted for 23.29 % of the total variation within the unplanted side of the macrocosm, with PC2 accounting for 18.29 % and PC3 for 11.22 % (Table 5.2). These first three principal components explained a total variation of 52.80 %. For the planted side of the macrocosm however, PC1 accounted for 33.57 %,

PC2 accounted for 11.86 % with PC3 accounting for 10.3 %. Hence for the planted side of the macrocosm the first three principal components accounted for 55.73 % for the total variation. Analysis of variance was carried out on PC scores, with amendment and fungal species as factors.

Table 5.2: Results of principal component analysis of fungal TRF relative abundance.
Data are for each treatment within the unplanted and planted sides of the macrocosms at the end of the investigation.

	PC1	PC2	PC3
Unplanted side			
Eigenvalues	558.7	438.7	269.2
% Variance	23.29	18.29	11.22
Cumulative	23.29	41.58	52.8
Planted side			
Eigenvalues	623.3	220.2	191.2
% Variance	33.57	11.86	10.3
Cumulative	33.57	45.43	55.73

Factor loadings describe which fragments contribute the most variation in the principal component analysis. Factor loading values were analysed for all fragments to ascertain which were making a significant contribution to PC1, PC2 and PC3 for both the unplanted and planted sides of the macrocosms. The fragments with the highest loading values in each PC axis were identified and any fragments with PC loading values > ± 0.25 were deemed significant (Pio *et al*., 1996).

For the **unplanted** side of the macrocosm (Figure 5.19) PC1 was influenced by the presence or absence of two fragments, PC2 determined by three fragments and PC3 by three fragments. For example PC1 shows that when a sample profile contains a fragment of length 103 bp, it does not contain one of 260 bp. The sample would

therefore have a positive PC1 score; if the PC1 score was negative, fragment length 260 bp would be present and 103 bp would not be found.

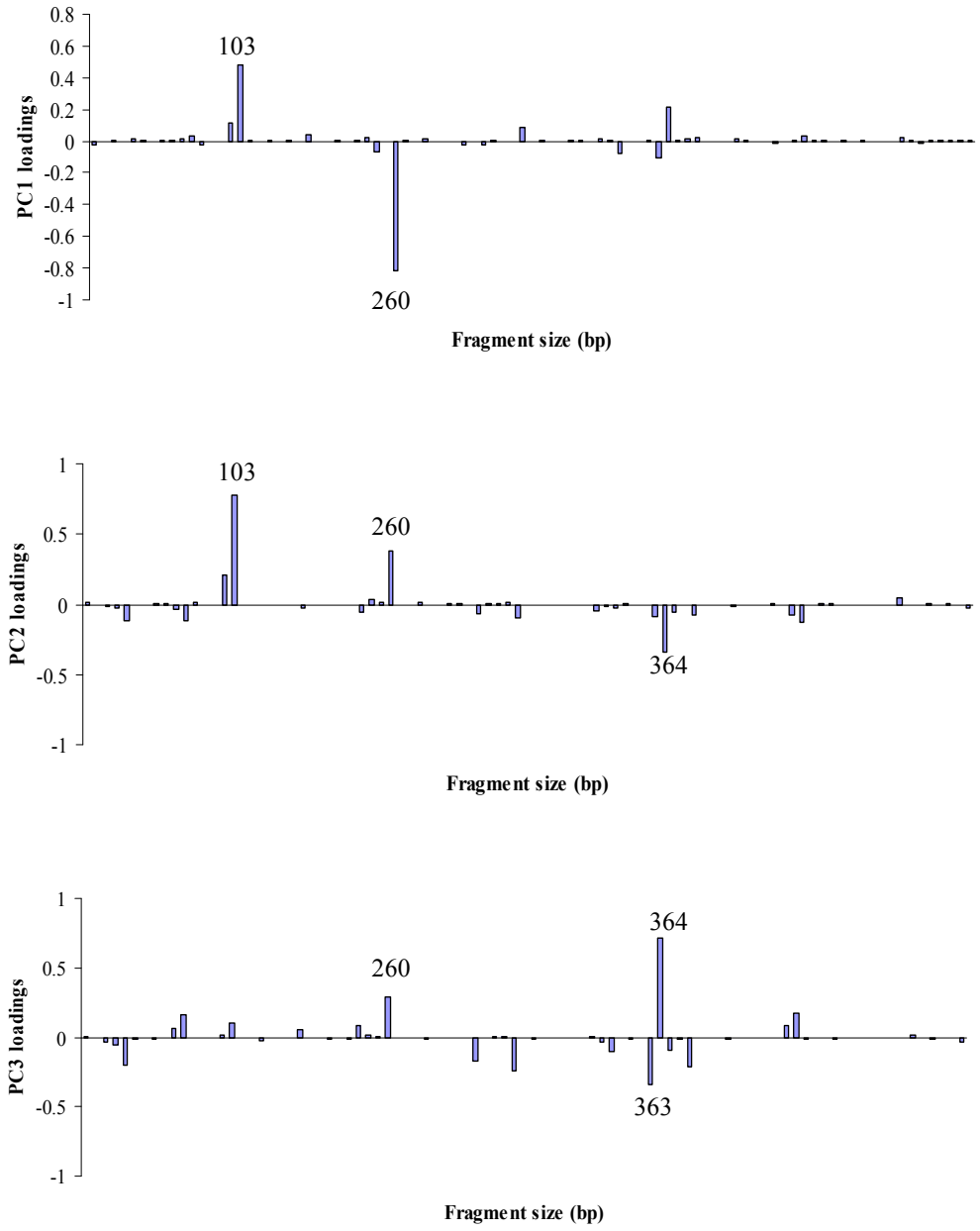


Figure 5.19: PC loadings for the first three principal components (PC) from relative abundance data collected from fungal T-RFLP analysis of soil from the unplanted side of the macrocosms.

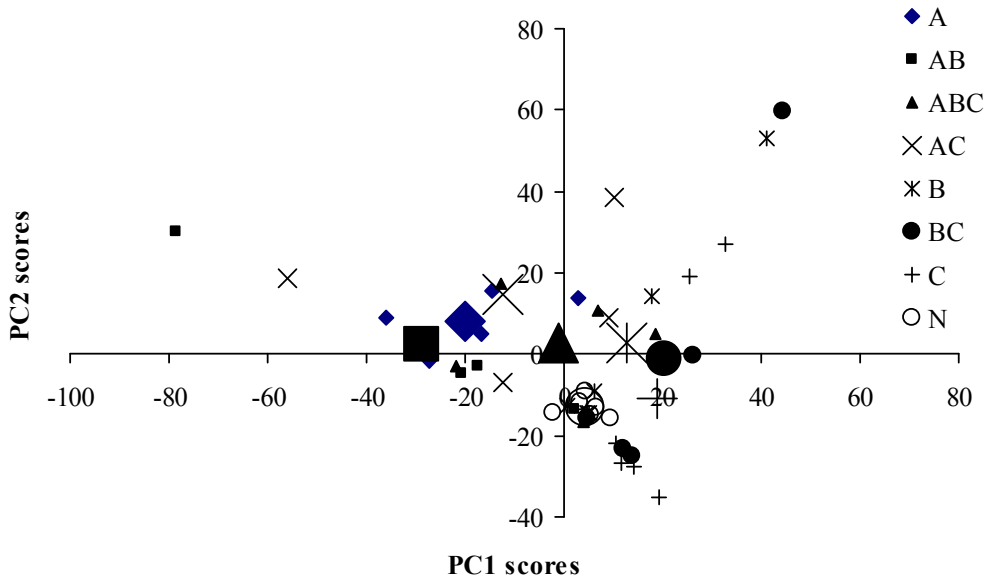


Figure 5.20: Ordination plot of PC1 scores versus PC2 scores for fungal T-RFLP fragments for each fungal species mixture within the unplanted side of the macrocosms.

Large symbols indicate centroids of mean PC scores. (Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

Figure 5.20 is an ordination plot of PC1 versus PC2 scores for all fungal species mixtures and for macrocosms amended or not, with the soil slurry (within the unplanted side). Microbial DNA profiles from the macrocosms containing *G. mosseae* and *G. intraradices* individually, when combined and also within the non-mycorrhizal planted treatment had positive PC1 scores (ANOVA of PC1 scores; fungal species mix, $F_{7,25} = 5.27$, $P < 0.001$), which was due to the presence of fragment 103 bp. In contrast macrocosms containing *G. geosporum* individually, *G. geosporum* plus *G. mosseae* and *G. geosporum* plus *G. intraradices* all had an extremely negative PC score reflecting the dominance of fragment 260 bp (this was also true for the three-species mix but not to the same degree, since the PC1 score was less negative).

Assessment of the PC2 scores suggested that soil slurry amendment had a significant effect on two AMF treatments (i.e. the macrocosms containing *G. mosseae* plus *G. intraradices* and *G. intraradices* individually) with the slurry supplemented macrocosms having a positive PC2 score compared to the unamended macrocosms. All remaining macrocosms had positive PC2 scores except non-mycorrhizal treatments (± soil slurry) and the amended macrocosms containing *G. geosporum* plus *G. mosseae* and *G. mosseae* individually (slurry x AMF interaction, $F_{7,25} = 2.58$, $P = 0.038$). The results suggest that within the supplemented macrocosm containing *G. intraradices* individually and when combined with *G. mosseae,* fragments of size 103 and 260 bp would be present with fragment 364 bp absent, whereas the reverse would be true when the PC2 scores are negative.

Ttreatments containing *G. intraradices* individually and *G. mosseae* in mixture, had significantly higher (and positive) PC3 scores in the unamended macrocosm compared to the amended (ANOVA of PC3 scores; slurry x AMF interaction, $F_{7,25} = 2.87$, $P = 0.024$). This suggests that these macrocosms are associated with the presence of fragments 260 and 364 bp and absence of fragment 363 bp (Table 5.3).

Table 5.3: Impact of soil slurry amendment (±) and AMF species mix on PC3 scores ($F_{7,25}$ = 2.87, P = 0.024)

AMF species	Slurry amended soil	Unamended soil
Sterilised	-ve (-20.82)	-ve (-16.36)
A	+ve (1.54)	-ve (-7.40)
AB	-ve (-7.84)	+ve (0.64)
ABC	+ve (12.05)	-ve (-5.16)
AC	+ve (7.25)	+ve (6.65)
B	-ve (-11.32)	-ve (-9.06)
BC	-ve (-4.20)	+ve (20.93)
C	+ve (6.09)	+ve (31.65)

Data are PC3 means. +ve PC3 scores reflect absence of fragment 363 bp and presence of 260 and 364 bp, with negative scores reflecting the reverse. (Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

For the **planted** side of the macrocosm, PC1 was influenced by the presence or absence of one fragment, PC2 by three fragments and PC3 by five fragments (Figure 5.21).

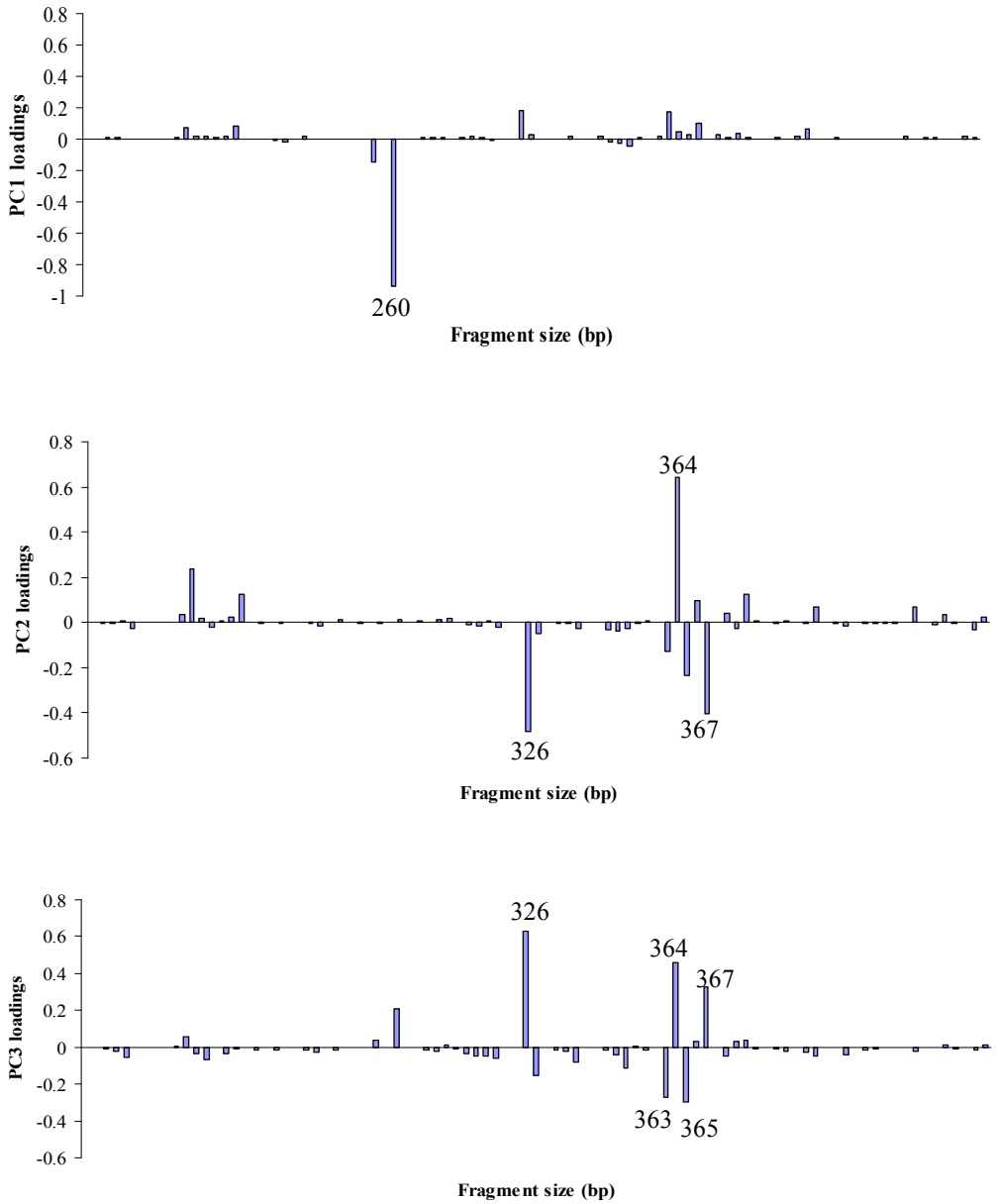


Figure 5.21: PC loadings for the first three principal components (PC) from relative abundance data collected from fungal T-RFLP analysis of soil from the planted side of the macrocosms.

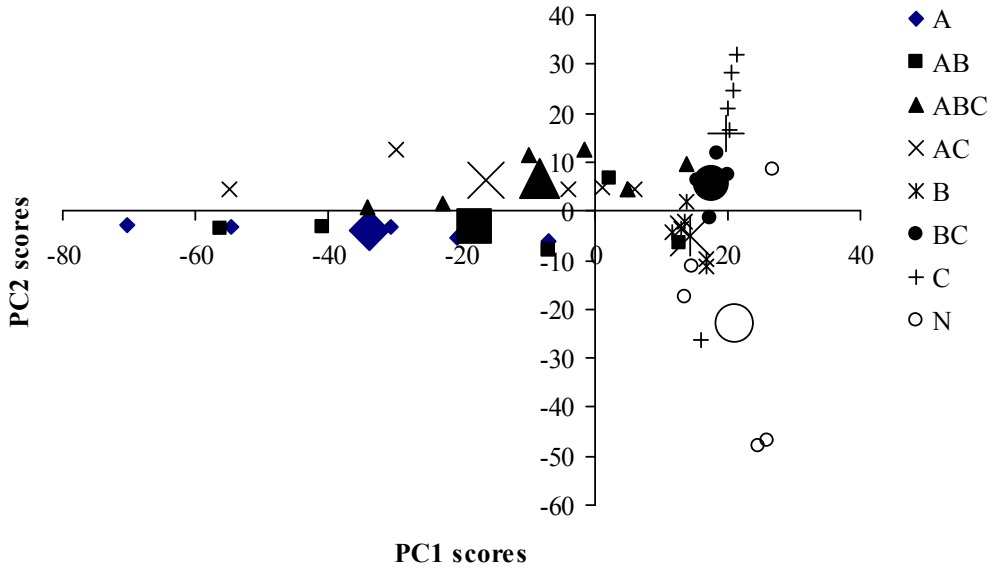


Figure 5.22: Ordination plot of PC1 scores versus PC2 scores for fungal T-RFLP fragments for each AMF species mixture within the planted side of the macrocosm. *Large symbols indicate centroids of mean PC scores. (Codes containing A, B and C relate to AMF species with A* = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

Figure 5.22 is an ordination plot of PC1 versus PC2 scores from all AMF species mixtures and soil amendments. Microbial DNA profiles from the planted side of all macrocosms containing *G. geosporum* (individually and in combination) had a negative PC1 score, due to the presence of fragment 260 bp (ANOVA of PC1 scores from the planted side of the macrocosm; AMF species mix, $F_{7,28} = 14.91$, $P < 0.001$). All remaining macrocosms had positive PC1 scores reflecting the absence of fragment 260 bp. All treatments containing *G. geosporum* except for the slurry amended macrocosms containing *G. geosporum* plus *G. intraradices* had a negative PC1 score. Macrocosms containing *G. geosporum* plus *G. mosseae* (-1.0), *G. geosporum* plus *G. intraradices* (1.07) and the non-mycorrhizal treatments (25.84) all had significantly higher PC1 scores in the slurry amended macrocosms than in the unamended ones. In macrocosms

without soil slurry, only those containing *G. geosporum* plus *G. intraradices* contained TRF 260 bp.

PC2 is based on the presence of TRF 364 bp and absence of TRFs 326, 364 and 367 bp in amended macrocosms inoculated with *G. geosporum*, *G. mosseae, G. geosporum* plus *G. mosseae* and sterilised inoculum. The remaining AMF mixes contained TRFs 326, 364 and 367 bp whilst 364 bp was absent (AMF single factor, $F_{7,28} = 5.50$, $P < 0.001$).

PC3 scores were affected by a slurry x AMF interaction ($F_{7,28} = 11.39$, $P < 0.001$; Table 5.4). Generally however all slurry supplemented macrocosms had a positive PC3 score (3.26), compared to those without amendment (-2.06) ($F_{1,28} = 5.41$, $P < 0.001$). PC3 was negative in columns without slurry amendment when with *G. intraradices* and *G. mosseae* individually and also in combination, in addition to the 3-species mix, reflecting the presence of fragments 363 and 365 bp as well as the absence of fragments 326, 364 and 367 bp. Within slurry amended macrocosms, fragments 326, 364 and 367 bp were present but 363 and 365 bp absent. Due to the limited identification database for fungal TRFs no presumptive identification of the fragments observed here could be made with the exception of fragment 103 bp that may have belonged to the *Thanatephorus* genus (Table 4.10; Dickinson, *pers. comm*., 2009).

Table 5.4: PC3 scores for fungal TRFs within the planted side of the macrocosms ($P_{7,28} = 11.39$, $P < 0.001$).

AMF species	Slurry amended soil	Unamended soil
Sterilised	+ve (36.88)	-ve (-18.44)
A	+ve (4.43)	-ve (-7.07)
AB	-ve (-7.11)	-ve (-4.00)
ABC	+ve (1.98)	-ve (-2.83)
AC	-ve (-1.08)	+ve (11.22)
B	-ve (5.10)	-ve (-12.03)
BC	-ve (-1.54)	+ve (3.76)
C	-ve (-2.40)	-ve (11.86)

Data are PC3 means. +ve PC3 scores reflect absence of fragment 363 and 365 bp and the presence of 326, 364 and 367 bp, with negative scores reflecting the reverse. (Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices)

5.3.7.5 Bacterial T-RFLP

For the bacterial data set PC1 accounted for 64.22 % of the total variation within the unplanted side of the macrocosm, with PC2 accounted for 11.22 % and PC3 accounted for 8.8 % (Table 5.5). These first three principal components explained a total variation of 84.24 %. For the planted side of the macrocosm however, PC1 accounted for 58.71 %, PC2 accounted for 14.62 % with PC3 accounting for 6.03 %. Hence for the planted side of the macrocosm the first three principal components accounted for 79.36 % for the total variation.

Table 5.5: Results of principal component analysis of bacterial TRF relative abundance for each treatment within the unplanted and planted sides of the macrocosms at the end of the investigation.

	PC1	PC2	PC3
Unplanted side			
Eigenvalues	1056.2	184.6	144.8
% Variance	64.22	11.22	8.8
Cumulative	64.22	75.44	84.24
Planted side			
Eigenvalues	629.8	156.9	64.7
% Variance	58.71	14.62	6.03
Cumulative	58.71	73.33	79.36

Factor loading values were analysed for all bacterial fragments to ascertain which were making a significant contribution to PC1, PC2 and PC3 for both the unplanted and planted sides of the macrocosms. As described previously, any fragments with PC loading values > ± 0.25 were deemed significant (Pio *et al*., 1996). For the **unplanted** side of the macrocosm (Figure 5.23) PC1 can be described as the presence and absence of two fragments, PC2 is determined by three fragments and PC3 by four fragments.

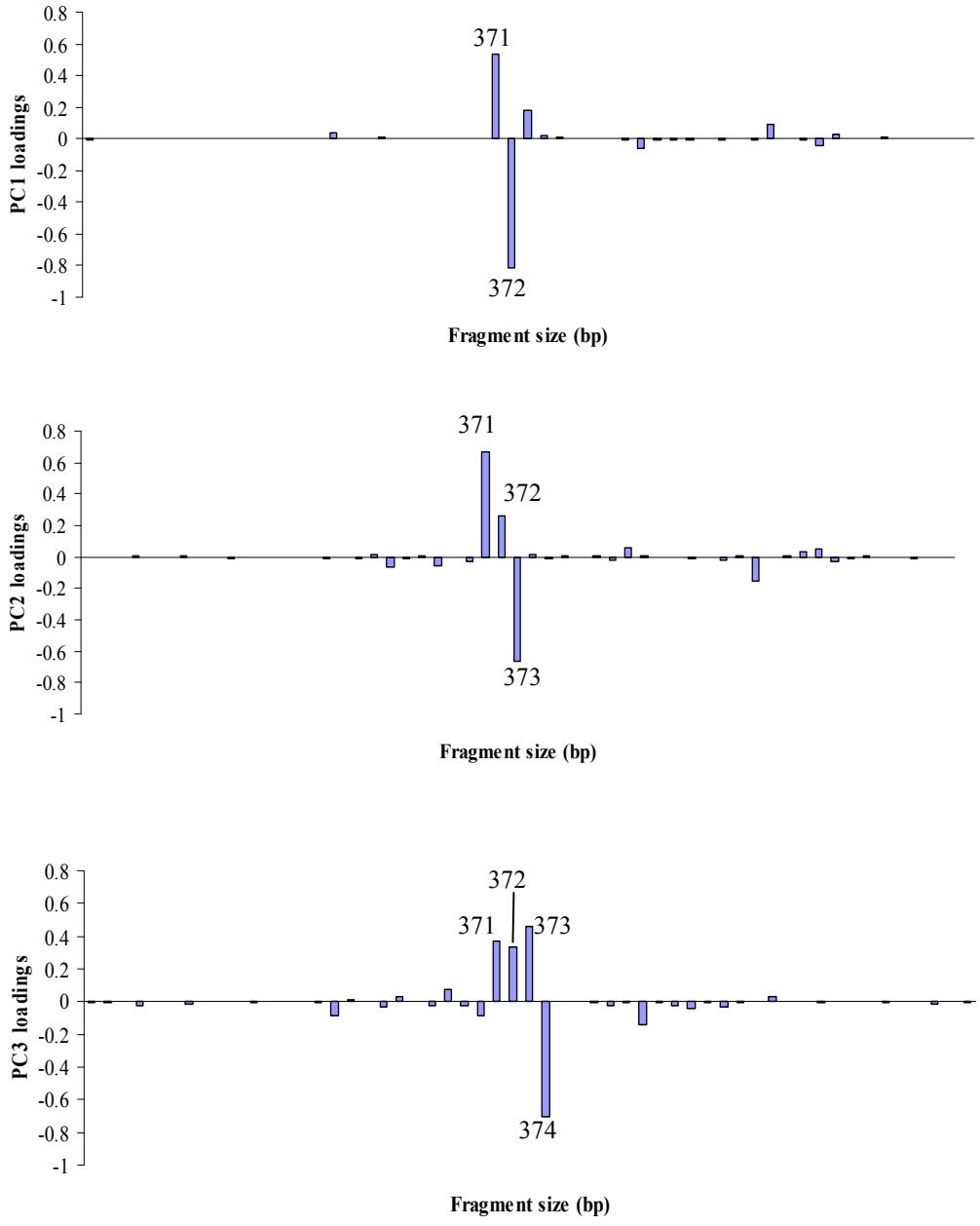


Figure 5.23: PC loadings for the first three principal component (PC) from relative abundance data collected from bacterial T-RFLP analysis of soil from the unplanted macrocosm side.

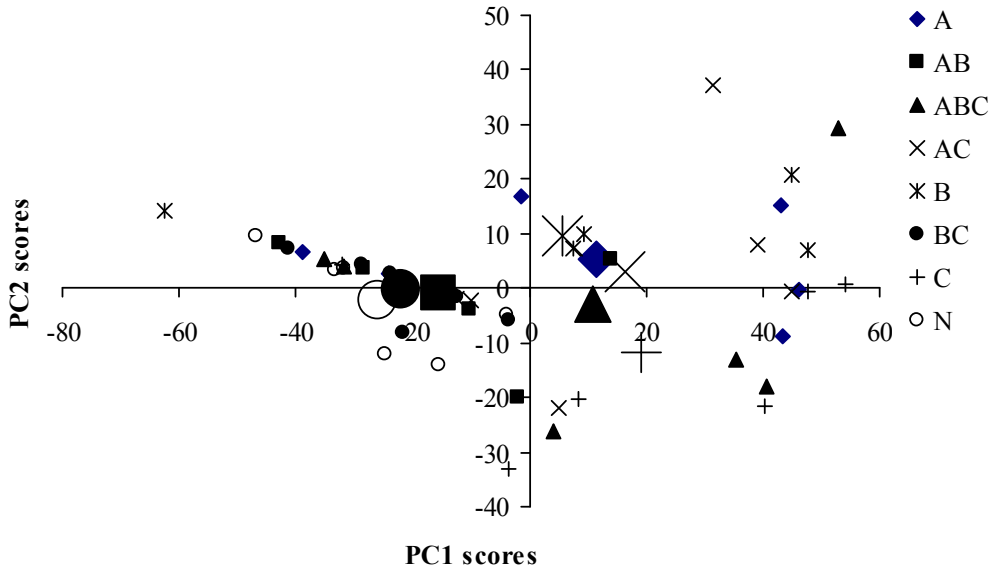


Figure 5.24: Ordination plot of PC1 versus PC2 scores for bacterial T-RFLP fragments for each fungal species mixture within the unplanted macrocosm side.
Large symbols indicate centroids of mean PC scores. (Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

Figure 5.24 is an ordination plot of PC1 versus PC2 scores from all AMF species mixtures and soil amendments. PC1 scores were positive (12.84) and negative (-12.84) in the slurry amended and unamended macrocosms respectively ($F_{1,30}$ = 13.95, P < 0.001), suggesting the presence of TRF 371 bp in the amended columns and the absence of TRF 372 bp in the unamended soils. Microbial DNA profiles from non-mycorrhizal macrocosms and those containing *G. geosporum* plus *G. mosseae* and *G. mosseae* plus *G. intraradices* had negative PC1 scores (-26.0, -15.46 and -21.94 respectively) ($F_{7,30}$ = 3.49, P = 0.007), which was due to the presence of fragment 372 bp and absence of fragment 371 bp. In the remaining macrocosms PC1 scores were all positive, reflecting the presence of fragment 371 bp and absence of 372 bp.

In slurry amended macrocosms containing the 3-AMF species mix, PC2 scores were negative (-18.97) but positive inthe unamended soils (12.89). The reverse was observed in the *G. geosporum* plus *G. intraradices* mix (with a PC2 score of 14.89 in the supplemented and -8.64 in the unamended macrocosms) (slurry x AMF interaction, $F_{7,30} = 2.83$, $P = 0.022$). Other supplemented macrocosms (those with *G. geosporum* plus *G. mosseae*, *G. mosseae* plus *G. intraradices*, *G. intraradices* individually and those with sterilised AMF inoculum) had negative PC2 scores reflecting the presence of fragment 373 bp, instead of TRFs 371 and 372 bp that would otherwise be associated with positive PC2 scores.

PC3 scores were not effected by added soil slurry or AMF species mix.

For the **planted** side of the macrocosm, PC1 and PC2 can be described as the presence or absence of three fragments, 371, 372 and 373 bp. PC3 is influenced by an additional fragment, 374 bp (Figure 5.25). For example PC1 shows that when a profile contains fragment of length 372 bp, it would have a negative PC1 score whereas if the PC1 score was positive fragment 372 bp would not be found and instead fragments 371 and 373 bp in length would be observed.

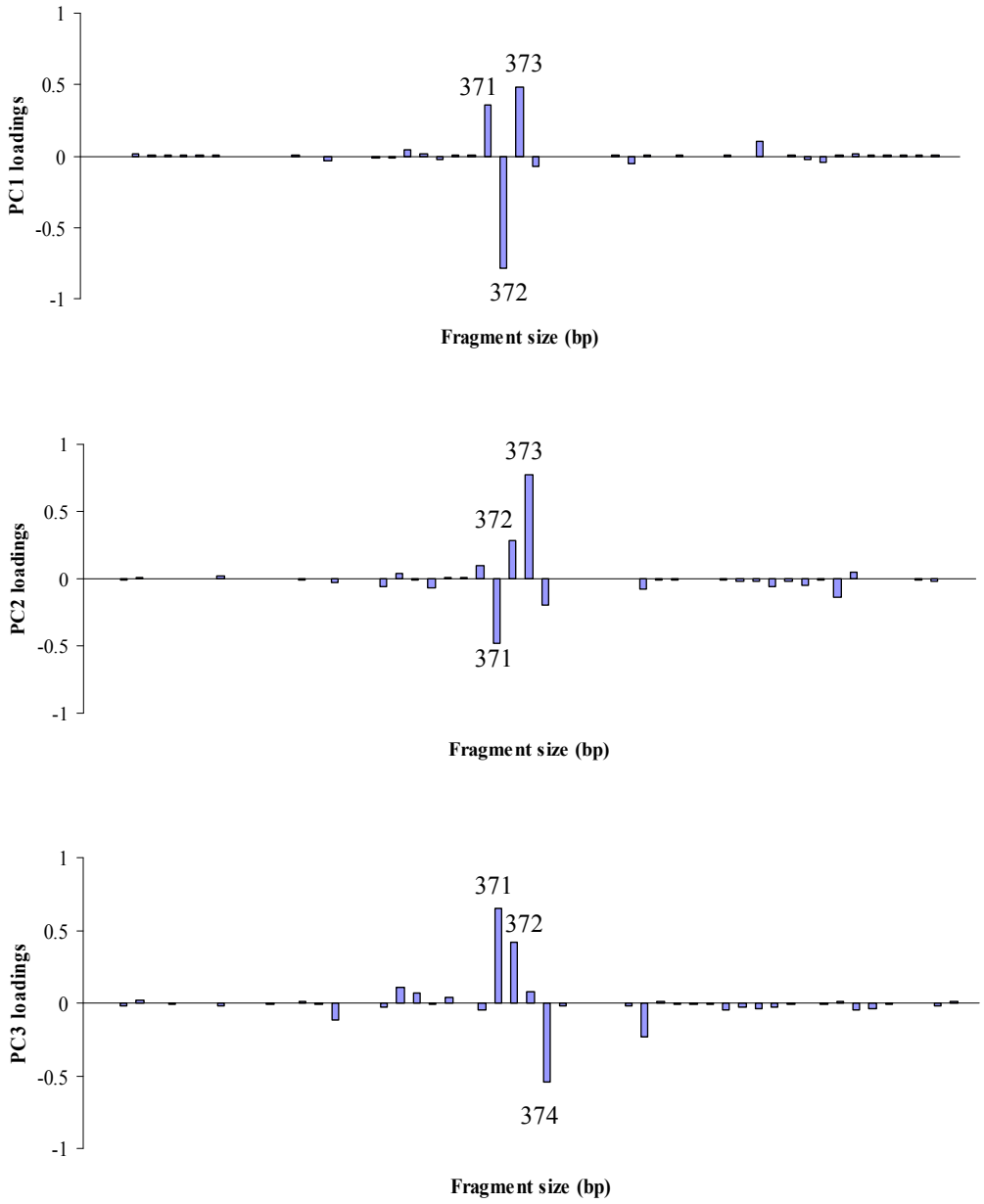


Figure 5.25: PC loadings for the first three principal components (PC) from relative abundance data collected from bacterial T-RFLP analysis of soil from the planted side of the macrocosms.

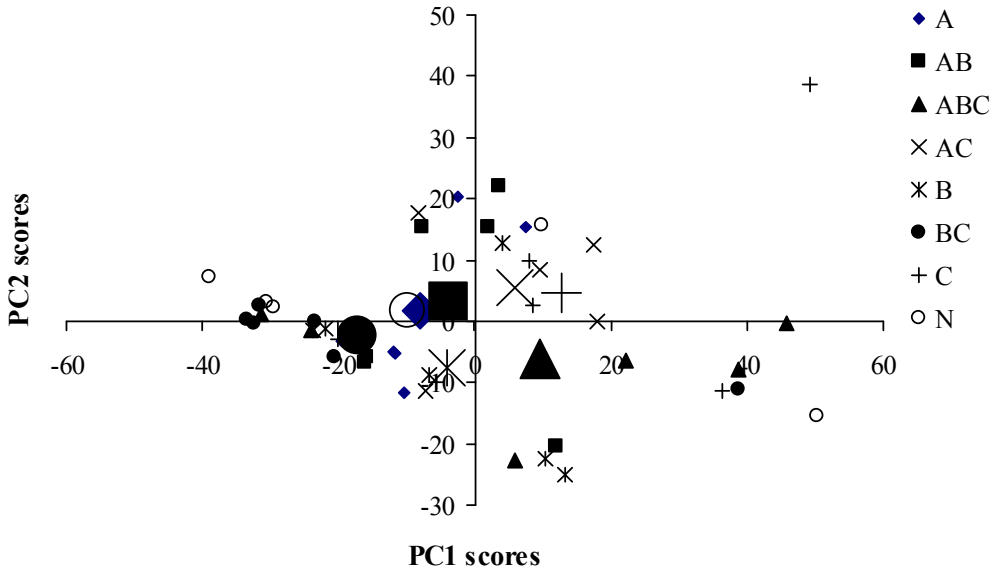


Figure 5.26: Ordination plot of PC1 scores versus PC2 scores for bacterial T-RFLP fragments for each AMF species mixture within the planted sides of the macrocosms. *Large symbols indicate centroids of mean PC scores. (Codes containing A, B and C relate to AMF species with A* = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

Figure 5.26 is an ordination plot of PC1 versus PC2 scores for all treatments. Neither AMF species mix nor slurry amendment significantly affected PC1 and PC2 scores derived from the planted side of the columns. However, slurry amended macrocosms had positive PC3 scores (1.55), compared to unamended columns which had negative scores (-2.18) ($F_{1,29} = 5.66$, $P < 0.0241$). Fungal species mixture also influenced PC3 scores within non-mycorrhizal macrocosms and within those containing *G. mosseae* plus *G. intraradices* and the 3-species mix; all having positive PC3 scores ($F_{7,29} = 4.45$, $P < 0.002$). This suggests the presence of TRFs 371 and 372 bp and absence of TRF 374 bp in the slurry amended soils. No slurry x AMF interaction was observed in the planted side of the macrocosms. Due to the limited identification database for bacterial TRFs using 23S rDNA, only fragment 371 bp could be presumptively identified as being from the *Burkholderia* genus (Table 4.10; Dickinson, *pers. comm*., 2009). Since

the close proximity of fragments 371, 372, 373 and 374 bp to each other, this suggests the fragments reflect the occurrence of a similar genus of bacteria.

5.3.8 AGGREGATE SIZE DISTRIBUTION

Aggregate size distribution (ASD) was unaffected by soil slurry amendment but differences between the planted and unplanted sides of the macrocosms were observed (Figure 5.27). Presence of roots resulted in a greater number of microaggregates (between 53 – 300 µm).

The planted side of the macrocosms had higher ASD_{CU} values (4.87) than the unplanted sides (4.03) (Figure 5.28; $F_{1,62} = 16.74$, $P < 0.001$). ASD_{CU} decreased with increasing number of AMF species present (Figure 5.29).

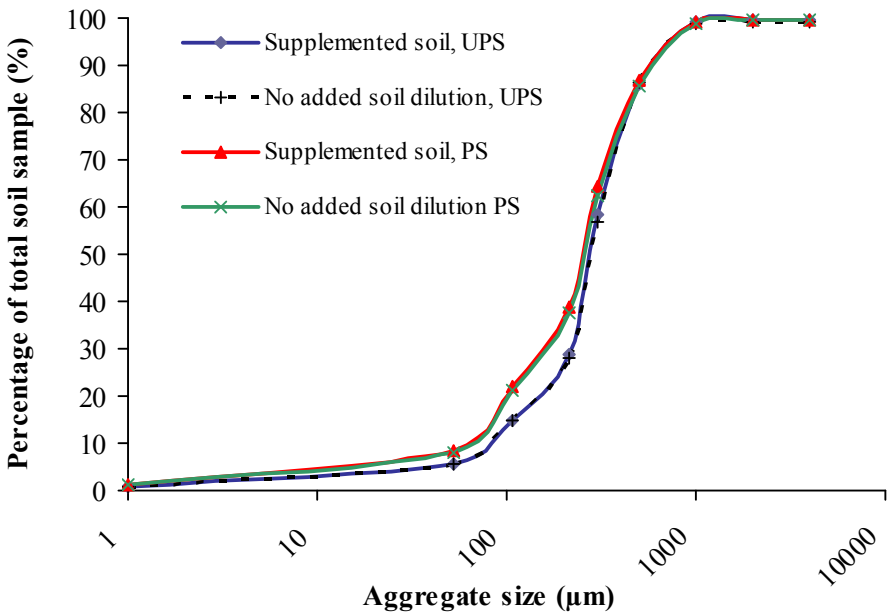


Figure 5.27: Aggregate size distribution for all each side of the macrocosm at each level of soil slurry amendment.
(Treatment codes: UPS = unplanted side of macrocosms, PS = planted side of macrocosm)

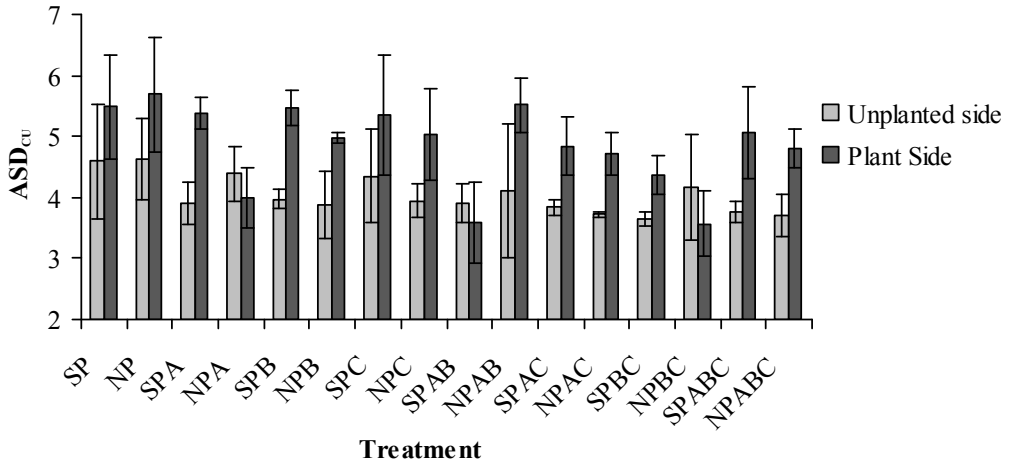


Figure 5.28: Coefficient of uniformity measurement from soil assessed either side of the split macrocosm under each treatment.

Data are means ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

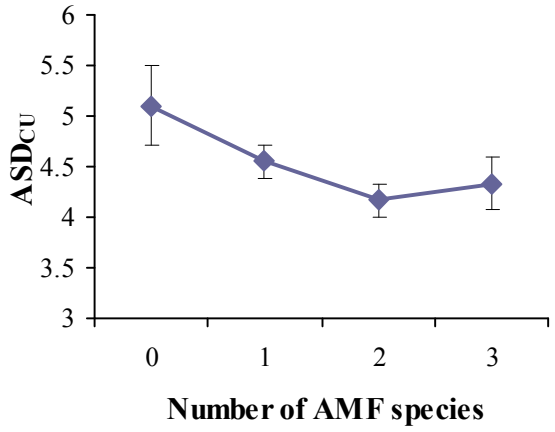


Figure 5.29: Effect of number of AMF species within the macrocosm on the coefficient of uniformity value of aggregate size data.

Data are means ± standard error. ANOVA of data shows a significant difference ($F_{3,90}$ = 2.72, P = 0.049).

5.3.9 AGGREGATE STABILITY

Aggregate stability was highest (classed as very stable) within the planted side of the macrocosm with a MWD of 2.23 mm compared to that of 1.61 mm within the

unplanted side (classed as stable) ($F_{1,62}$ = 47.40, P < 0.001; Figure 5.30). The amendment with soil slurry at the start of the investigation had a significant impact on aggregate stability, regardless of the side of the macrocosm analysed ($F_{1,62}$ = 4.29, P = 0.042). Amended soil macrocosms had a higher MWD value of 2.01 mm, compared to 1.82 mm for the unamended macrocosms. Neither AMF species combinations nor number of AMF species present affected aggregate stability.

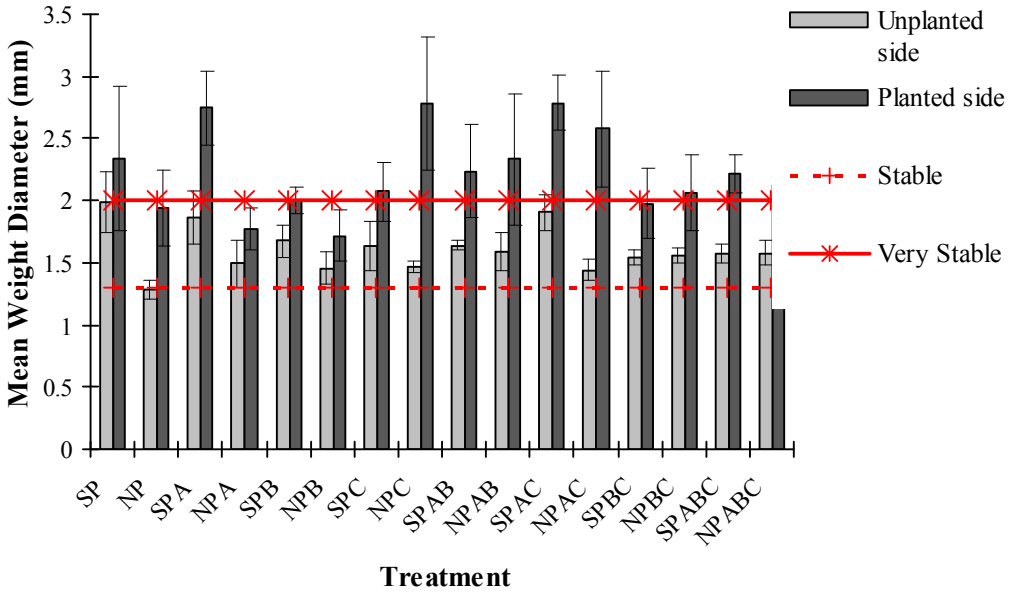


Figure 5.30: Aggregate stability given as mean weight diameter (in mm) within each macrocosm.

Data are means ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

5.3.10 Mesoscale Visual Evaluation of Soil Structure

Figure 5.31 show images used for pore size and morphological determination. Visual assessment of these images allows the changes in pore space (such as size and distribution) within the soil macrocosms to be observed. Porosity appeared to be higher within the planted side of the macrocosms at the start of the investigation (i.e. at seed

sowing) for all treatments within the supplemented macrocosms except for the treatment containing *G. mosseae* individually (where porosity was similar across both sides of the macrocosm) and the *G. geosporum* plus *G. intraradices* mix where porosity was highest within the unplanted side (Figure 5.31). Within the unamended (slurry) treatments containing each of the three *Glomus* species individually, a higher porosity within the planted side of the macrocosm compared to the unplanted side was observed. Within all other unamended treatments, porosity was higher within the unplanted side of the macrocosm.

After seven weeks incubation, porosity was generally highest within the planted side of the macrocosm for three of the treatments containing the 10^1 soil dilution (namely macrocosms containing *G. mosseae* individually, *G. geosporum* plus *G. mosseae* and the 3-species mix). Within the unamended soil macrocosm, all treatments except *G. mosseae* individually and the sterilised AMF inoculum, had lower porosity within the planted side of the macrocosm than the unplanted.

Within 5 treatments: i) Sterilised AMF inoculum; ii) *G. geosporum*, iii) the 3-species mix, all plus slurry amendment; iv) *G. geosporum* and v) *G. mosseae*, both minus amendment, porosity increased over the seven week period within the unplanted soils. The remaining treatments generally exhibited decreases in total porosity due to soil settling after packing, apart from amended macrocosms containing *G. intraradices* where porosity remained constant. Porosity decreased over time within the planted side of the macrocosm, within treatments containing the sterilised inoculum, *G. geosporum* individually, *G. geosporum* plus *G. mosseae*, the 3-species mixture, *G. intraradices*

individually (within the supplemented macrocosms) and within *G. geosporum* plus *G. intraradices* in the unamended soils. The remaining macrocosms, except the supplemented macrocosms containing *G. geosporum* plus *G. intraradices* and the unamended macrocosms with sterilised AMF inoculum, all showed an increase in porosity over time within the planted side of the macrocosm.

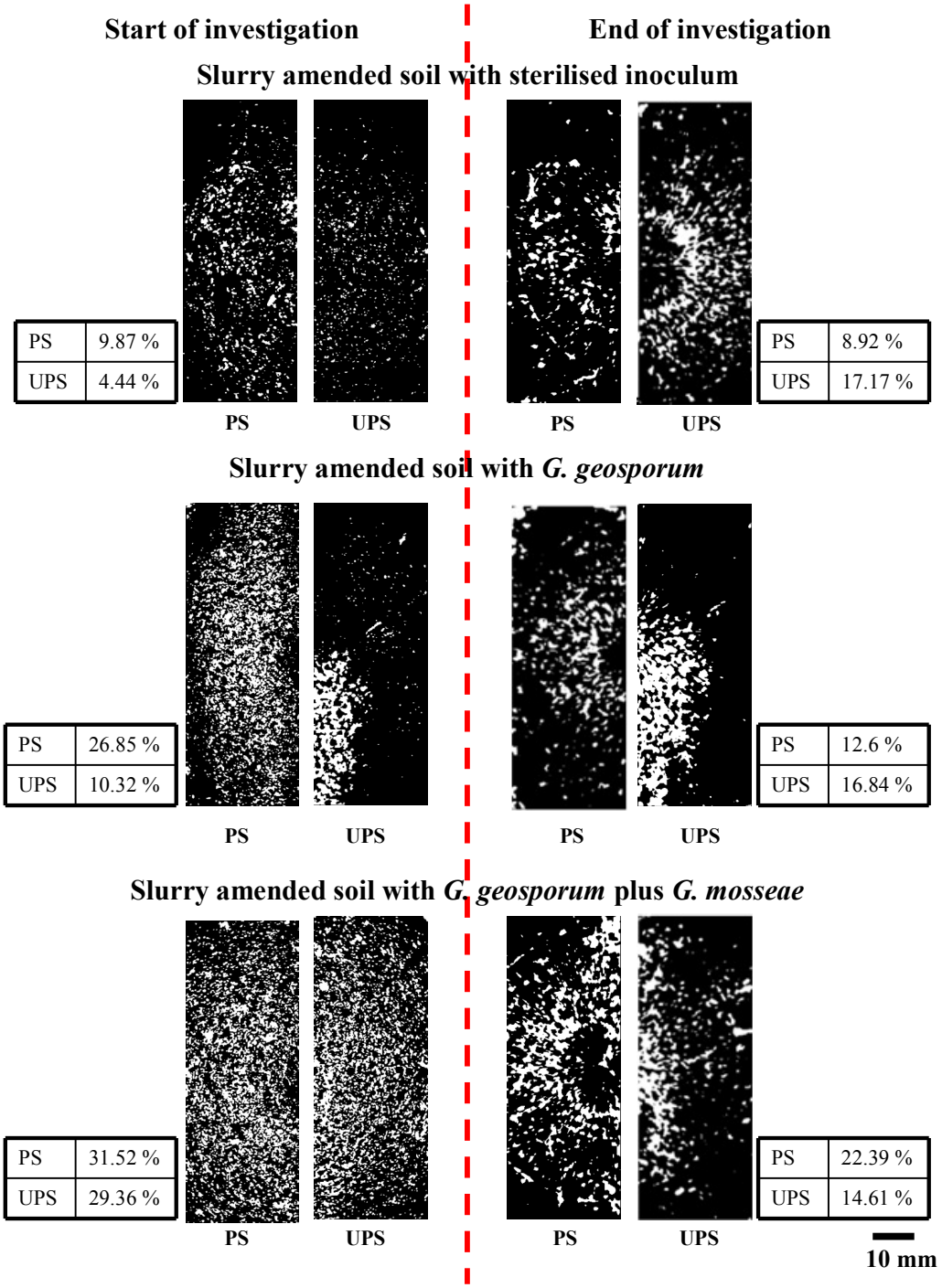


Figure 5.31: ***Continued***

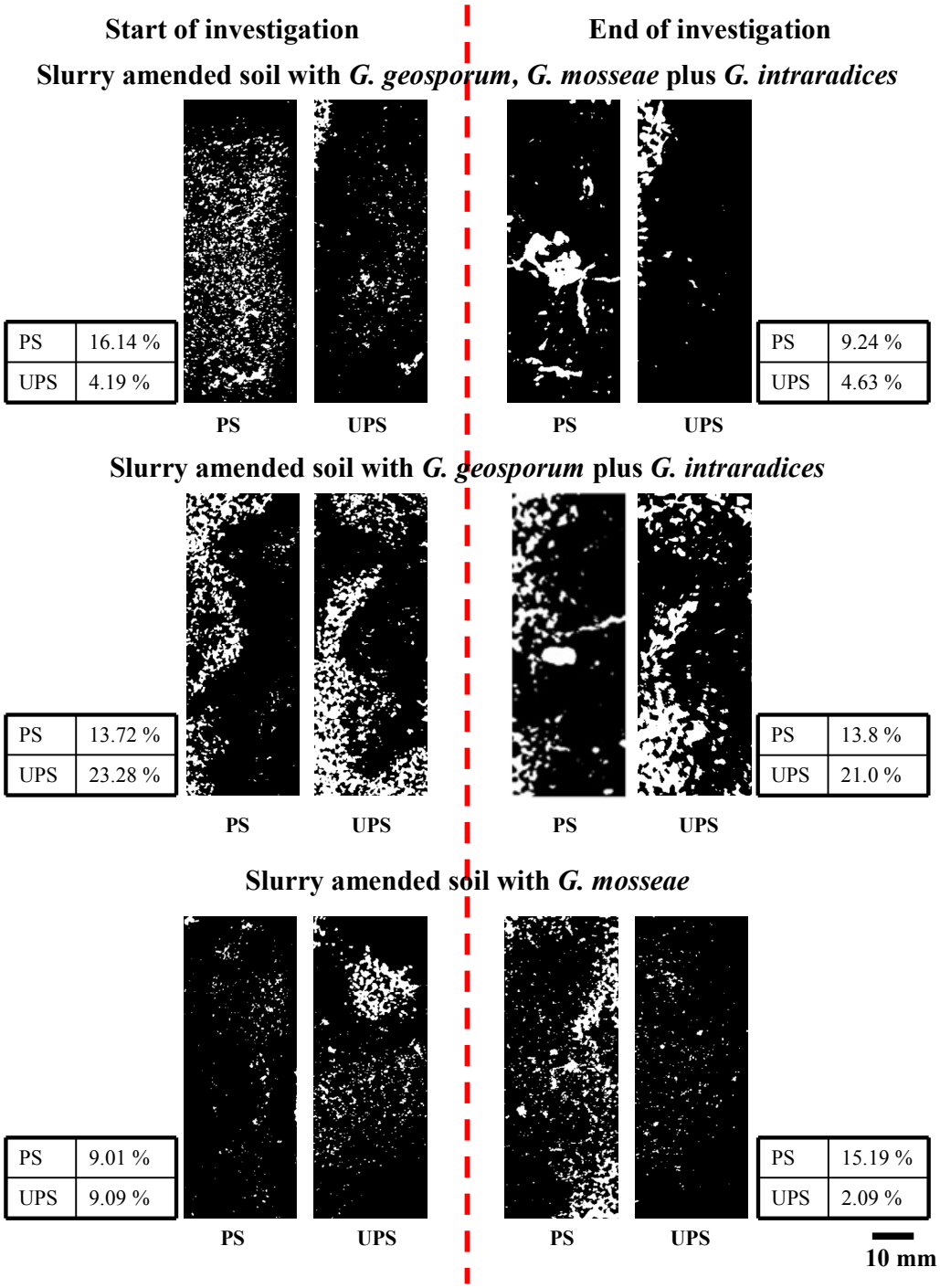


Figure 5.31: ***Continued***

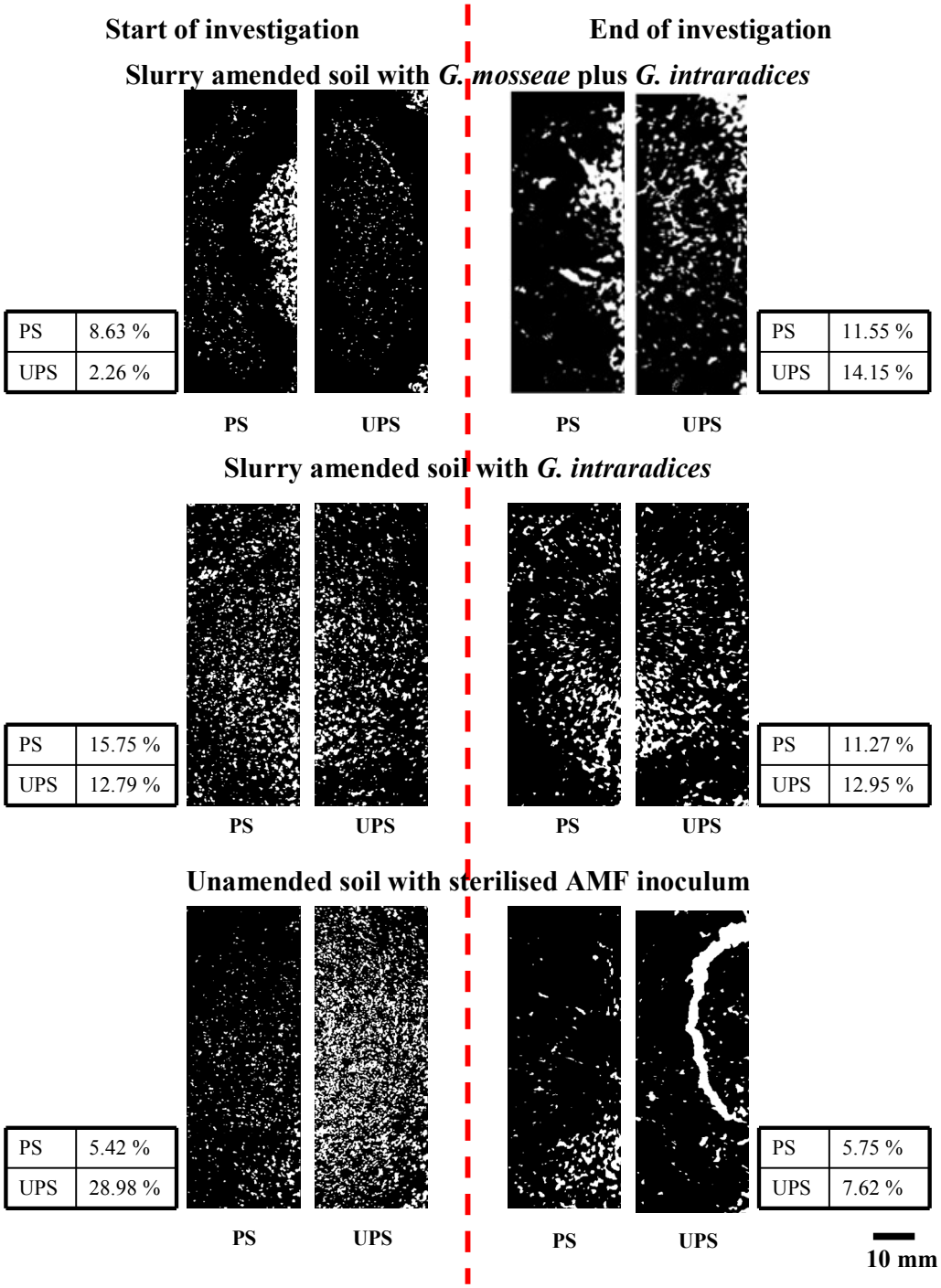


Figure 5.31: ***Continued***

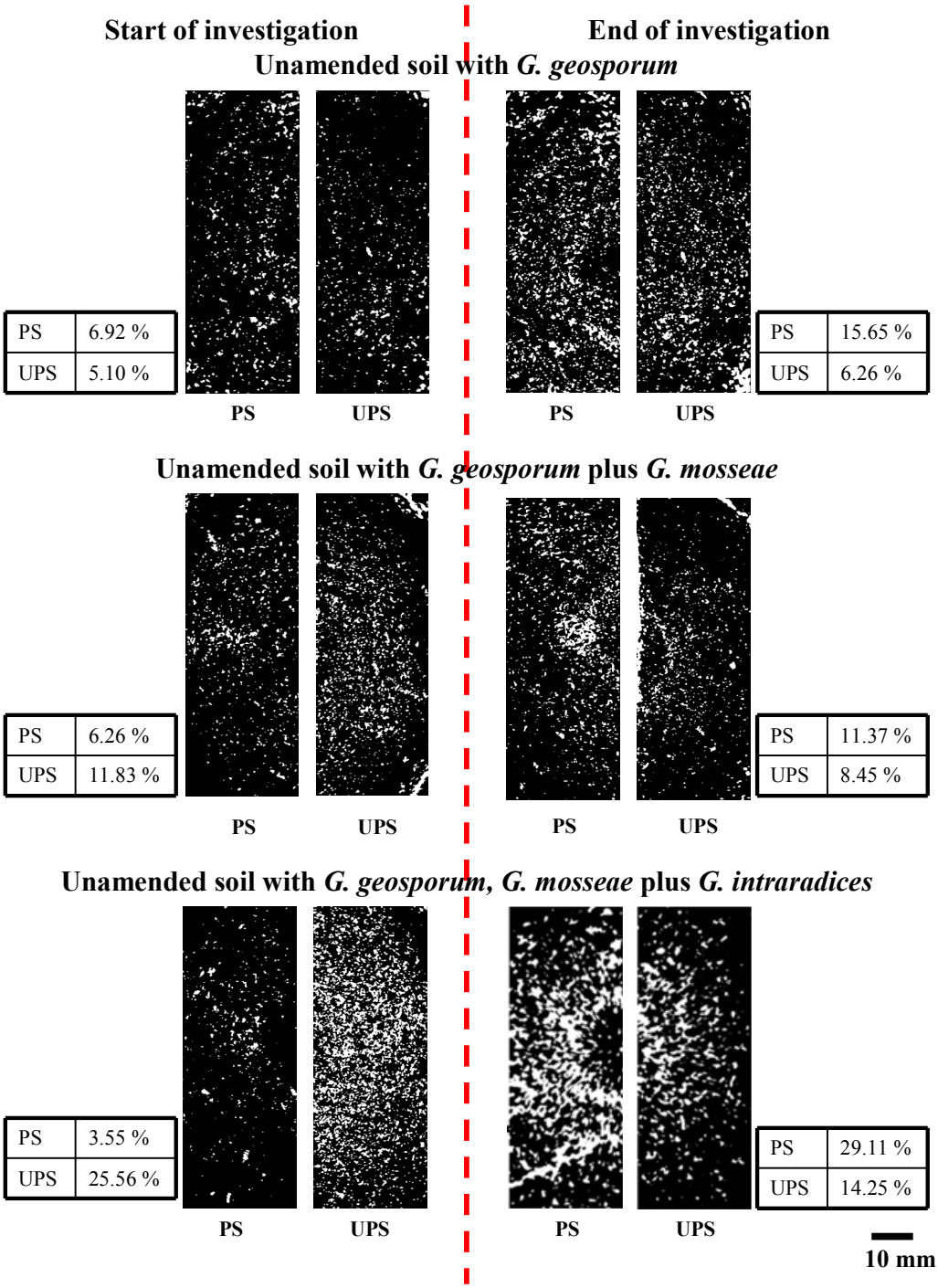


Figure 5.31: ***Continued***

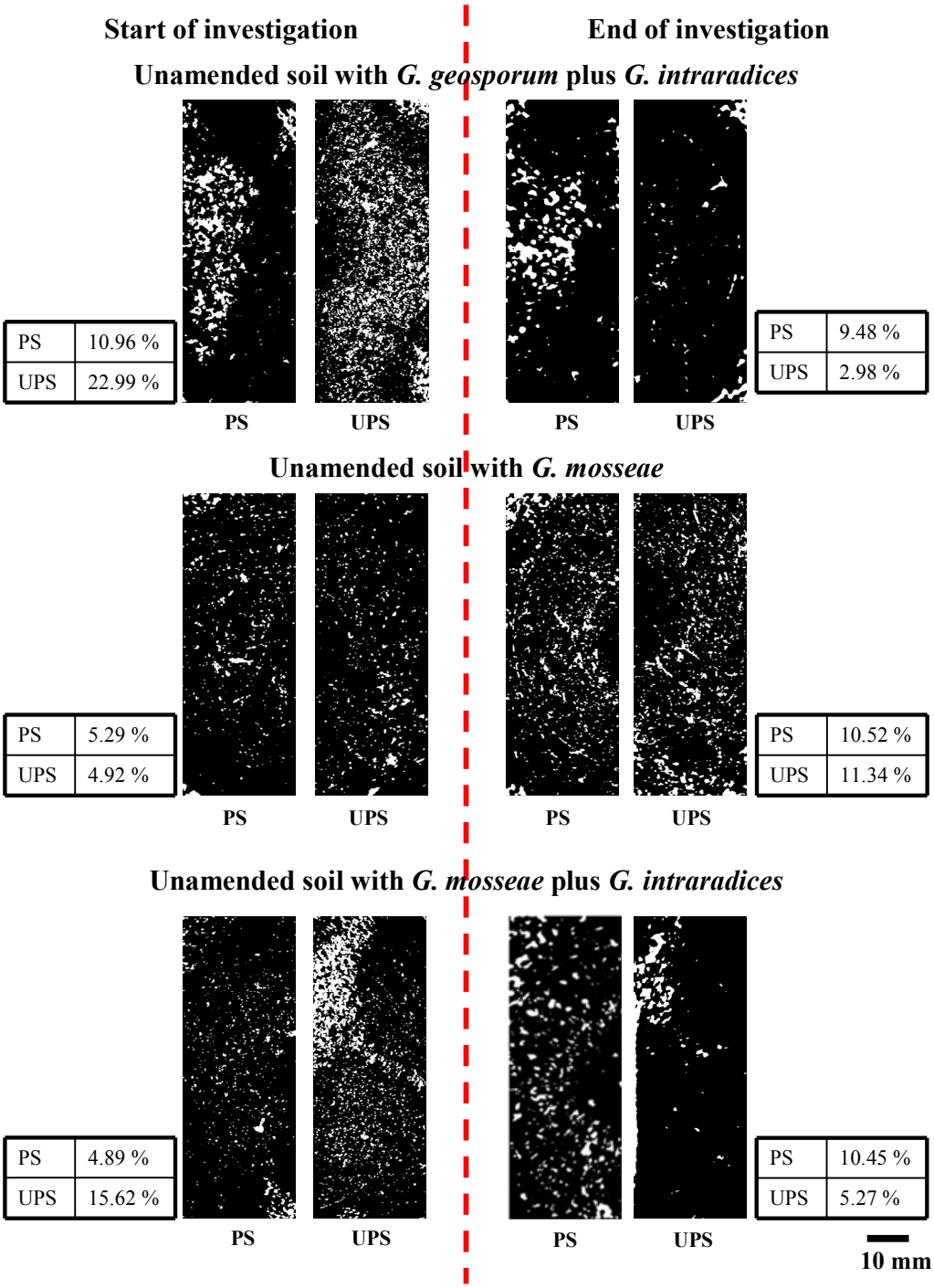


Figure 5.31: ***Continued***

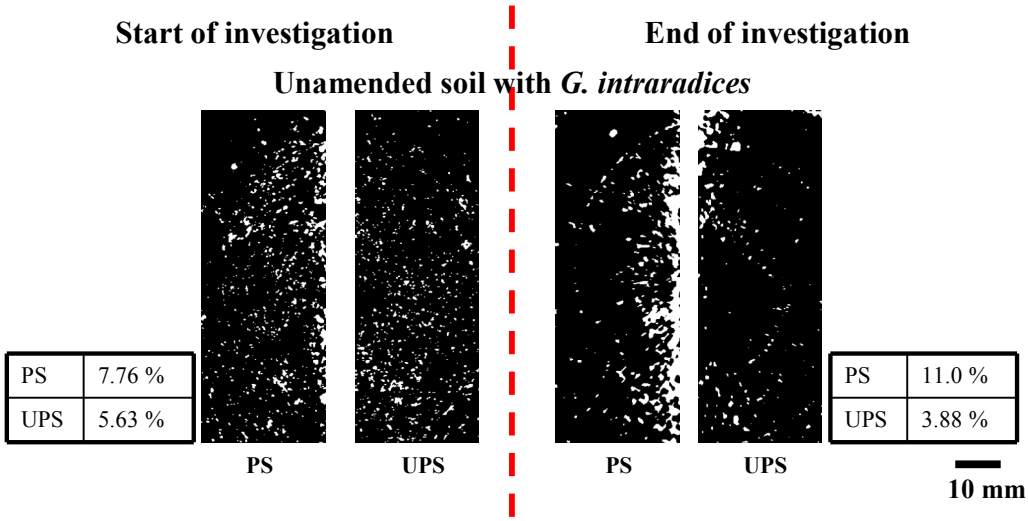


Figure 5.31: Example of processed images taken from X-ray CT for all treatments at both the start and end of the investigation.

Note: Pore space in images is represented in white. All figures given beside images reflect porosity values of that image. The same slice for each treatment (i.e. plant side (PS) and unplanted side (UPS) at each time period) is used that is representative of the mean total porosity of the treatment in question. Slurry amended and unamended refers to addition or omission of the soil dilution treatment. Start of investigation refers to the seedling stage.

5.3.11 TOTAL POROSITY

Total porosity was highest (12.92 %) within the supplemented macrocosms compared to the unamended macrocosms (10.03 %) (slurry amendment as a single factor, $F_{1,155}$ = 9.40, P = 0.003). Porosity was also highest within the planted side of the macrocosm (12.73 %) compared to that of the unplanted side (10.21 %) (split macrocosm effect; $F_{1,155}$ = 7.22, P = 0.008). An AMF x time interaction (Table 5.6) was observed in which the *G. geosporum* plus *G. intraradices* mix and the 3-species mix, had greater porosity at the start of the experiment compared to at the end (Figure 5.32). In contrast, porosity increased from 8.4 % to 14.4 % in the soils containing *G. mosseae* over the experimental period. As AMF species number increased within the soil macrocosms, porosity development decreases at the final harvest (Figure 5.33).

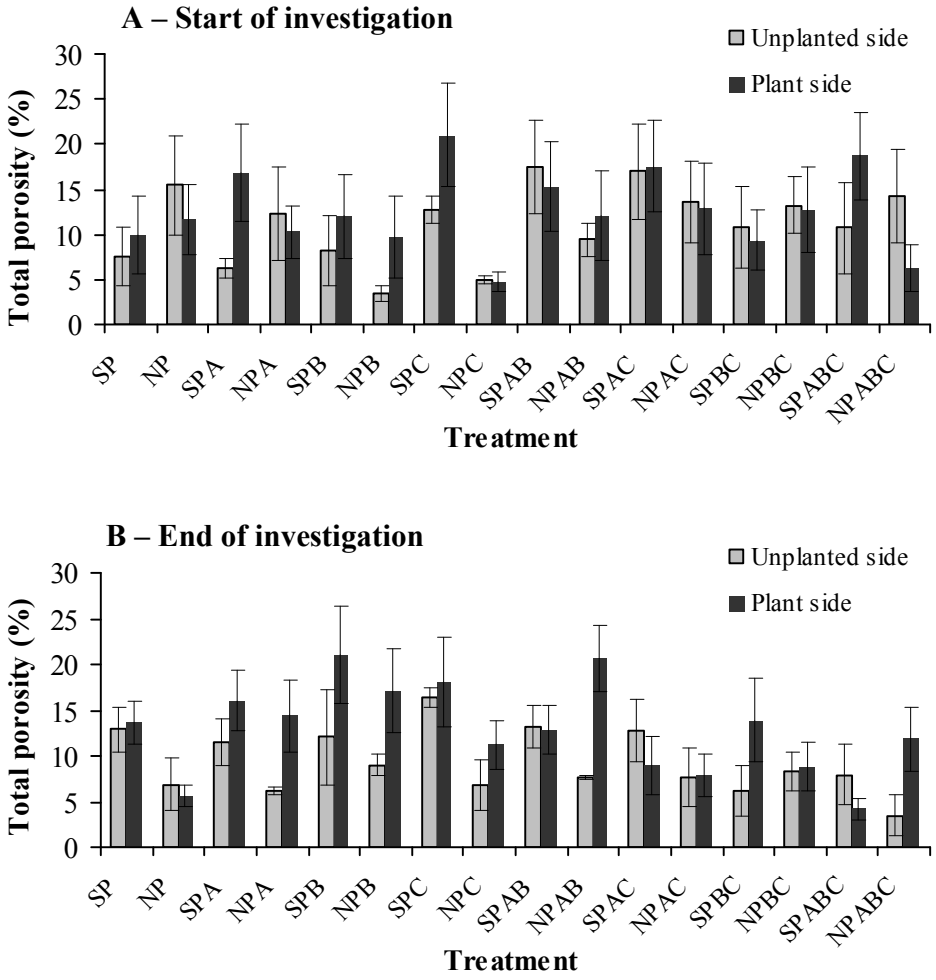


Figure 5.32: Total porosity with treatment and side within the split macrocosm at the A) start of the investigation and B) end of the investigation.

Data are means ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices*).*

Table 5.6: Results from repeated measurement ANOVA for total porosity.

Source of variation	DF	F	P
Slurry amendment	1	9.40	0.003
Split macrocosms side (planted or unplanted)	1	7.22	0.008
Residual	*155*		
Time x AMF species	7	2.61	0.014
Time x slurry amendment x split macrocosm side	1	4.65	0.033
Residual	*160*		

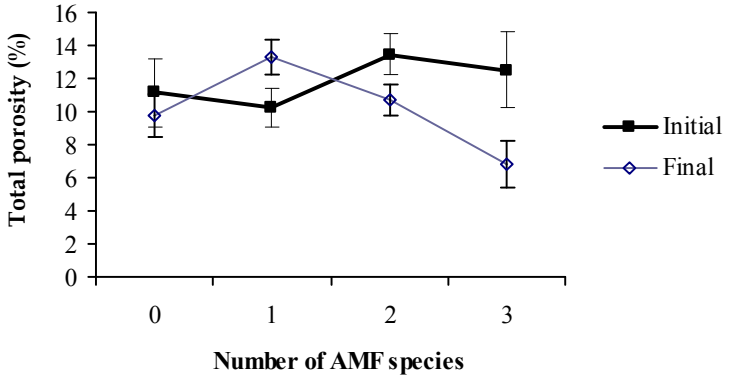


Figure 5.33: Effect of number of AMF species within the macrocosms on the total porosity.

Data are means ± standard error. ANOVA of data taken from the final harvest shows a significant difference ($F_{3,183}$ = 4.30, P = 0.006).

5.3.12 MEAN PORE SIZE

Mean pore size was greater within the slurry amended soils than in those without amendment (1.63 mm^2 and 1.52 mm^2 [log_{10}+2] respectively) (slurry amended as a single factor; Table 5.7). In addition, location within the split macrocosm influenced mean pore size, with larger pores observed in the plant side of the macrocosm compared to the unplanted (1.61 mm^2 and 1.55 mm^2 [log_{10} +2] respectively) ($F_{1,155}$ = 3.93, P = 0.049).

Mean pore size was greatest within amended soils *G. intraradices* (1.80 mm^2 [log_{10}+2]) and *G. geosporum* (1.75 mm^2 [log_{10}+2]) individually and in combination (1.76 mm^2 [log_{10}+2]); in addition to within the *G. mosseae* plus *G. intraradices* mix (1.68 mm^2 [log_{10}+2]). The lowest mean pore space was found within the macrocosms containing *G. mosseae* (1.30 mm^2 [log_{10}+2]) (AMF x slurry interaction (Table 5.7). Despite this, no impact of the number of AMF species and mean pore area was observed.

Table 5.7: Results from repeated measurement ANOVA for mean pore area.

Source of variation	DF	F	P
Slurry amendment	1	14.01	< 0.001
AMF species	7	7.01	< 0.001
Split macrocosms side (planted or unplanted)	1	3.93	0.049
Slurry amendment x AMF species	7	2.89	0.007
Residual	*155*		
Time	1	236.33	< 0.001
Time x AMF species	7	4.32	< 0.001
Time x slurry amendment x split macrocosm side	7	5.77	< 0.001
Residual	*160*		

Time of sampling also had a significant effect on mean pore area (Table 5.7) with pores larger at the end of the investigation than at the start (1.77 mm^2 and 1.63 mm^2 [log_{10}+2] respectively), highlighting an increase in mean pore size over time (Figure 5.34).

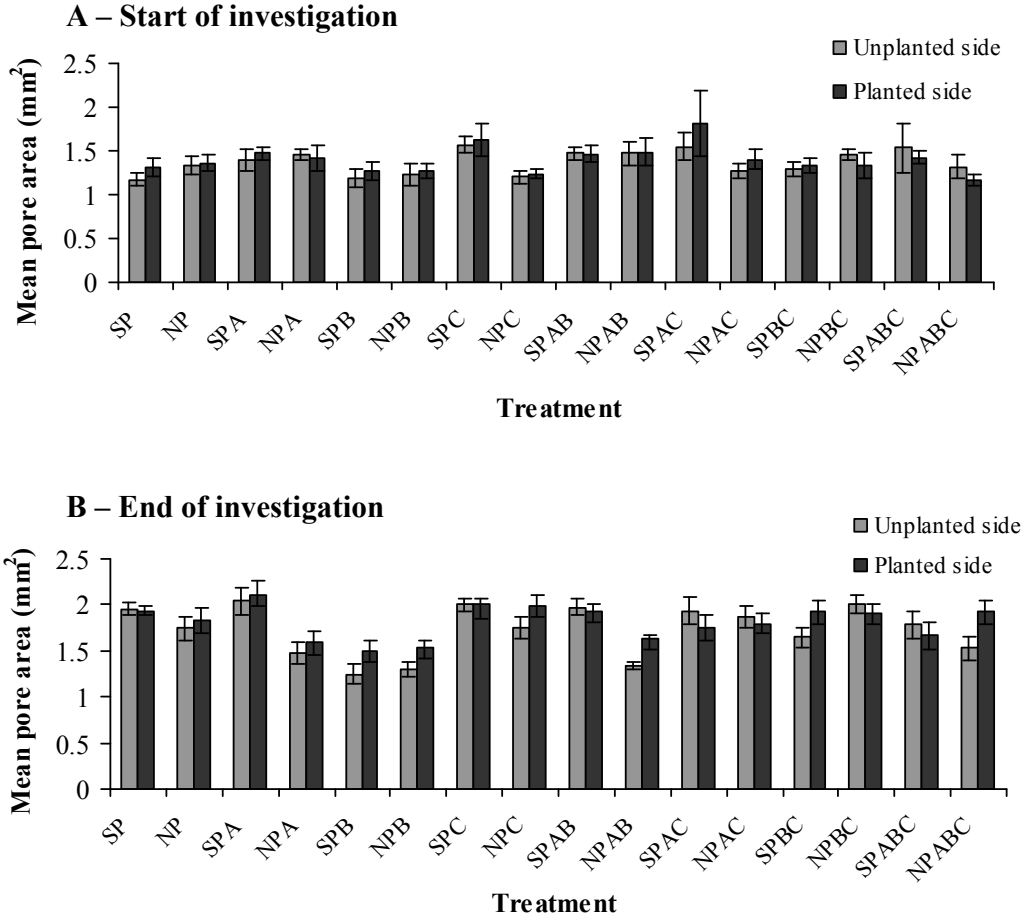


Figure 5.34: Mean pore area determined from image analysis of X-ray CT images at A) the start and B) the end of the investigation.
Data are means (log_{10}+2) ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

5.3.13 PORE SIZE DISTRIBUTION

Pore > 64 µm were quantified; pore size distributions were location dependent (Figure 5.35). In the slurry amended non-mycorrhizal treatment there was an increase in larger pores (31.6 – 100 mm^2 in size) within the planted side of the column in comparison to the unplanted side. This was not however the case in the unamended non-mycorrhizal macrocosms.

PSD was similar in both sides of the macrocosm in amended soil containing *G. mosseae* plus *G. intraradices,* and within the unamended macrocosms containing *G. intraradices* and the 3-species mix. In slurry amended treatments containing *G. mosseae*, *G. intraradices* and *G. geosporum* individually and *G. geosporum* plus *G. intraradices,* an increase in the number of large pores was observed in the planted side of the macrocosm. Within the unamended slurry treatments containing *G. geosporum* and *G. mosseae* individually, *G. geosporum* plus *G. mosseae* and *G. geosporum* plus *G. intraradices* the number of pores of 31.6 – 100 mm^2 in size increased.

At the initial analysis a higher percentage of pores within each pore size grouping were observed relative to the unamended macrocosms. Within the slurry amended treatments containing *G. geosporum* plus *G. intraradices* and the 3-species mix, there were significantly higher percentages of pores between 100-1000 mm^2 in size, suggesting areas of high pore connectivity within the macrocosms.

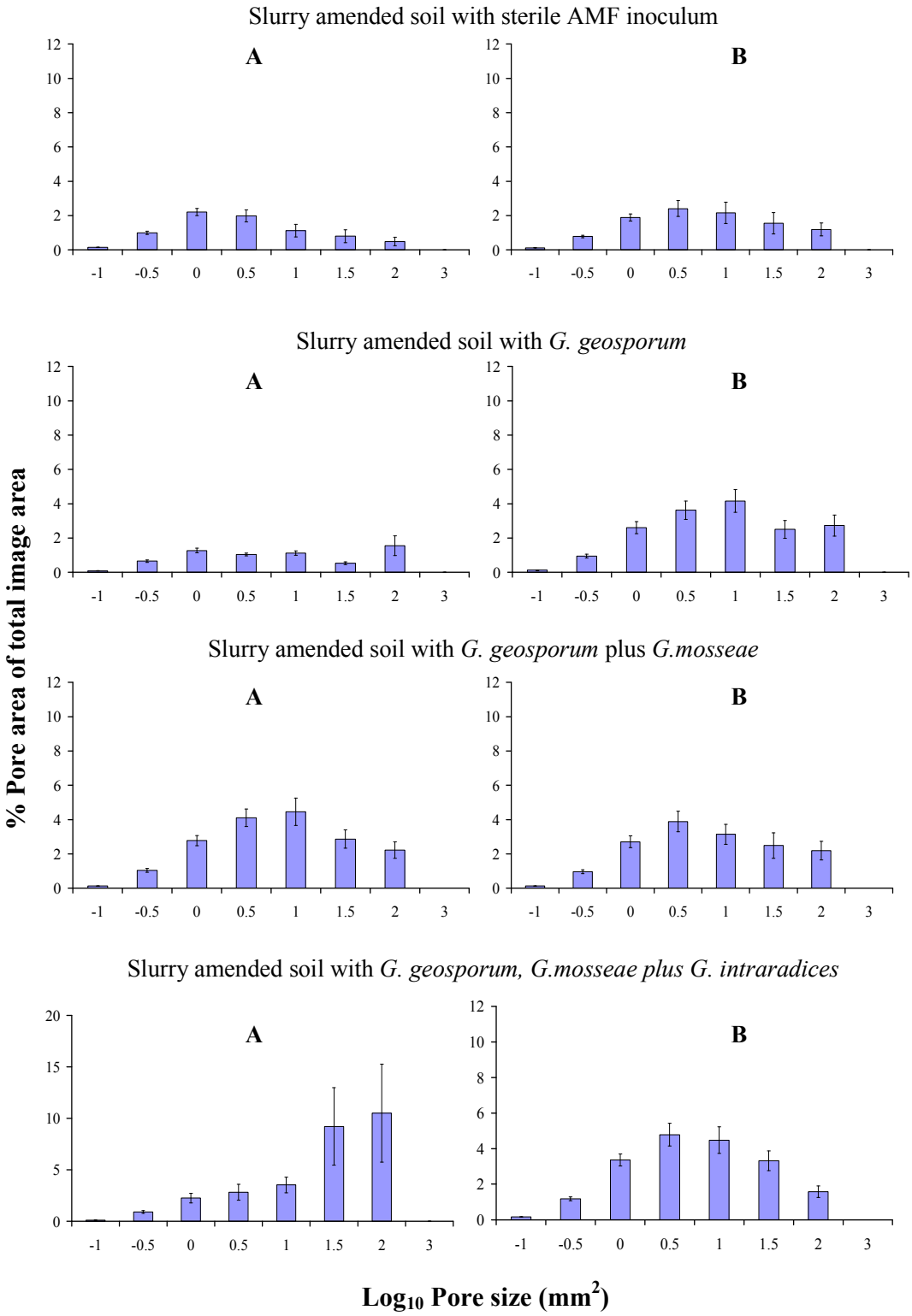


Figure 5.35: *Continued*

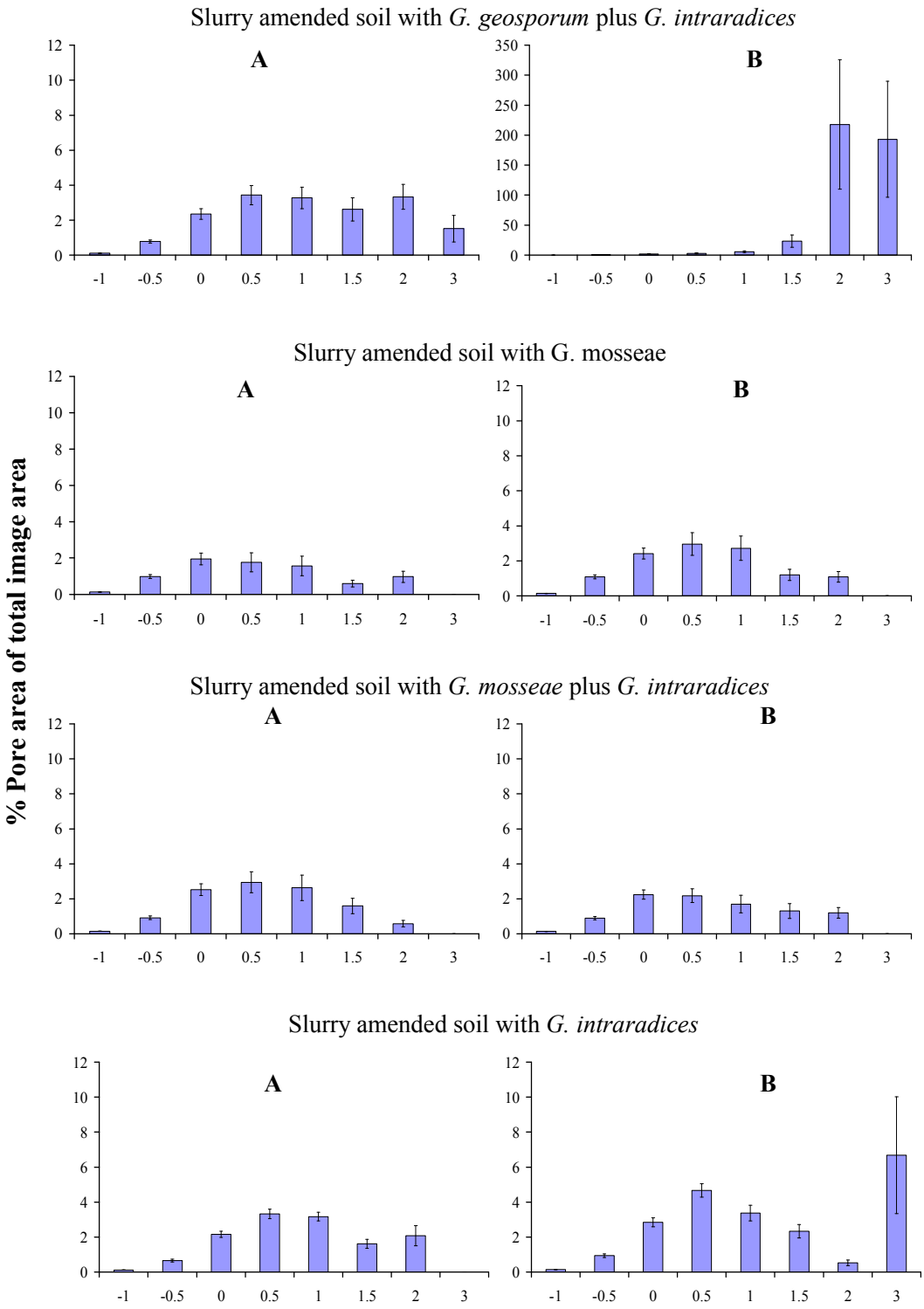


Figure 5.35: *Continued*

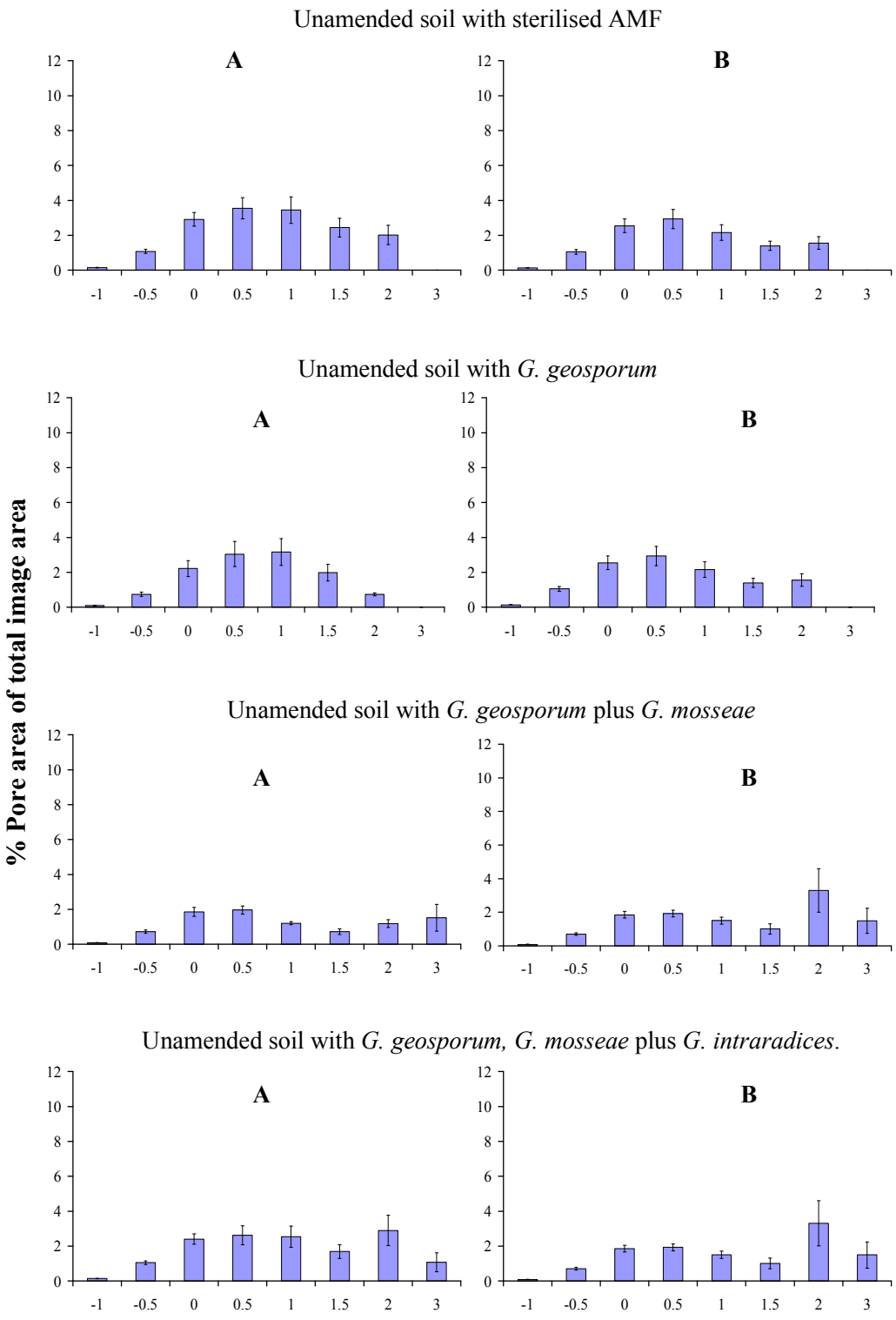


Figure 5.35: *Continued*

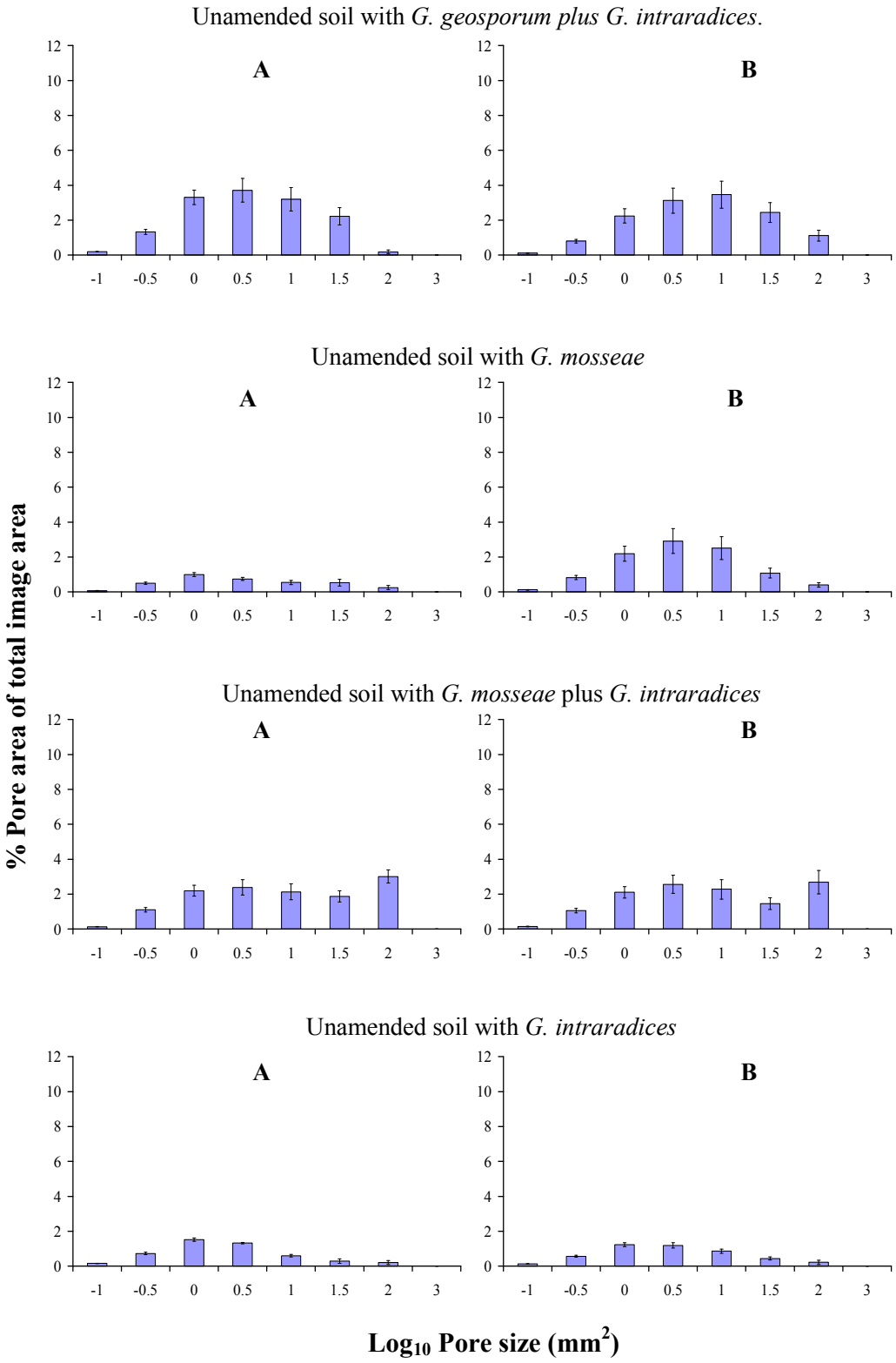


Figure 5.35: Pore size distributions from A) unplanted and B) planted sides of the macrocosms at the start of the investigation (at the seedling stage).
Data are means ± standard errors.

PSD was significantly influenced by location within the macrocosm, particularly after the seven week growth period (Figure 5.36. Within the slurry amended treatments containing *G. mosseae* and *G. intraradices* both individually and in combination, either a high percentage of pores or the presence of larger pores (> 1000 mm^2) was observed in the planted side of the column. Within the unamended treatments, location had a dramatic effect on PSD. In the treatments containing *G. mosseae*, *G. geosporum, G. intraradices*, *G. geosporum* plus *G. mosseae* and the 3-species mix, the presence of larger pores (>100-1000 mm^2) suggested an increase in pore connectivity. It was therefore apparent that the effect of location within the macrocosm (i.e. the presence of roots) was more important within the unamended macrocosms with respect to PSD.

Interestingly in both slurry amended and unamended soils, *G. geosporum* plus *G. intraradices* resulted in a low occurrence of larger pores suggesting the two species had a negative effect on PSD. Slurry amendment in non-mycorrhizal columns resulted in more pores of 3.16–100 mm^2 than in unamended soil. In the mycorrhizal soils, slurry amendment increased the percentage of pores within all pore size classes in the unplanted side of the columns; this effect was lost in the planted side.

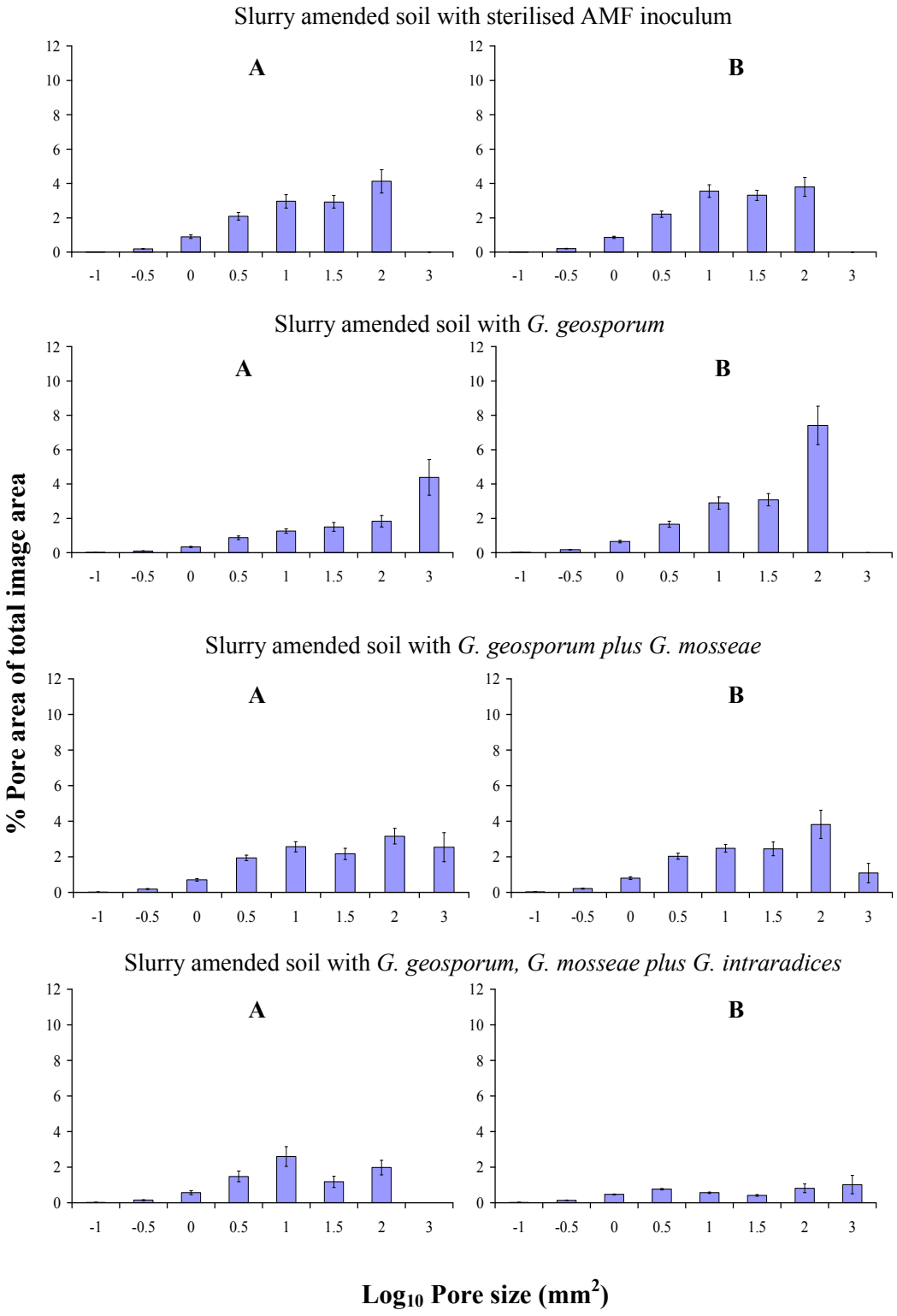


Figure 5.36: ***Continued***

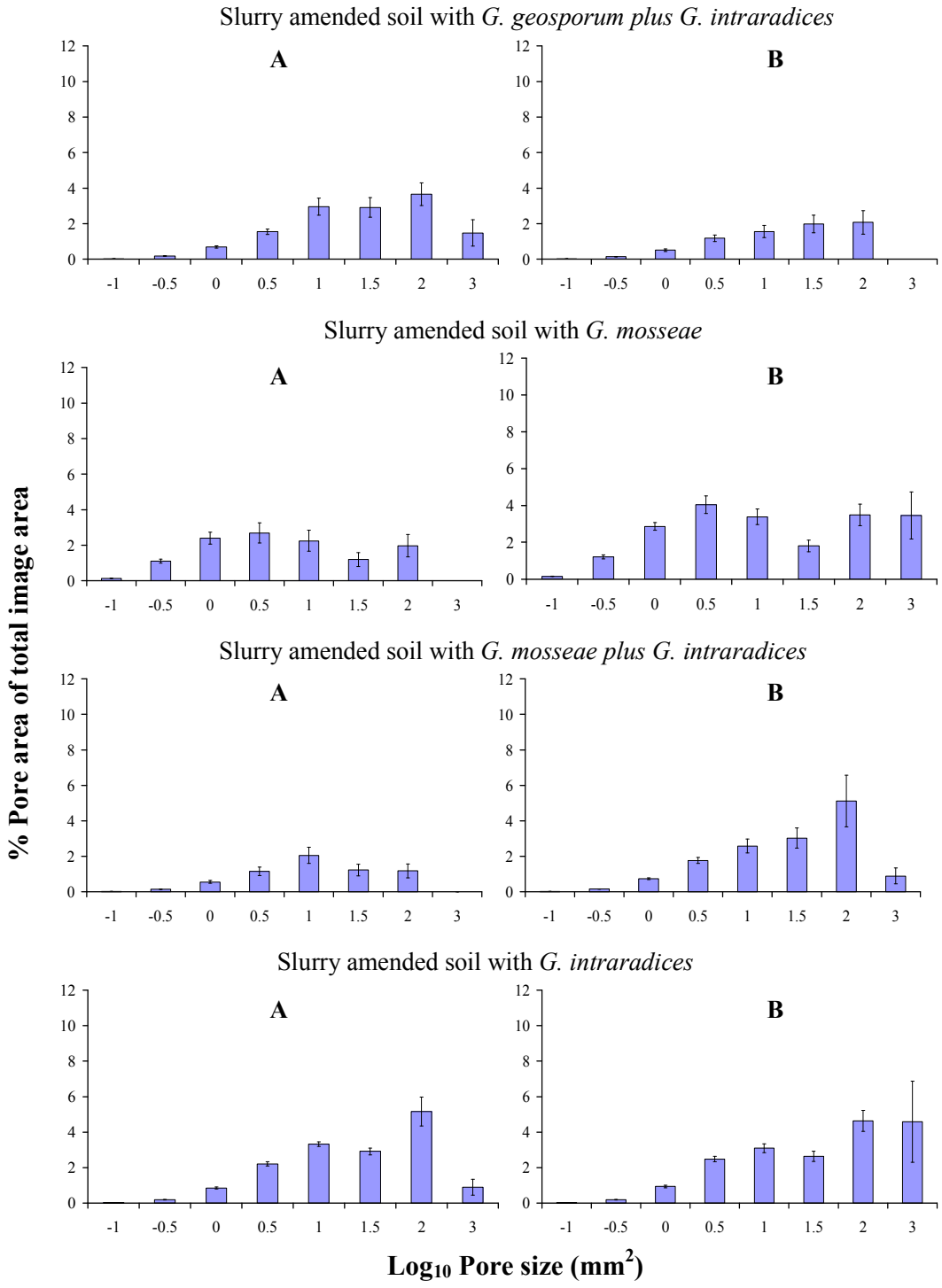


Figure 5.36: *Continued*

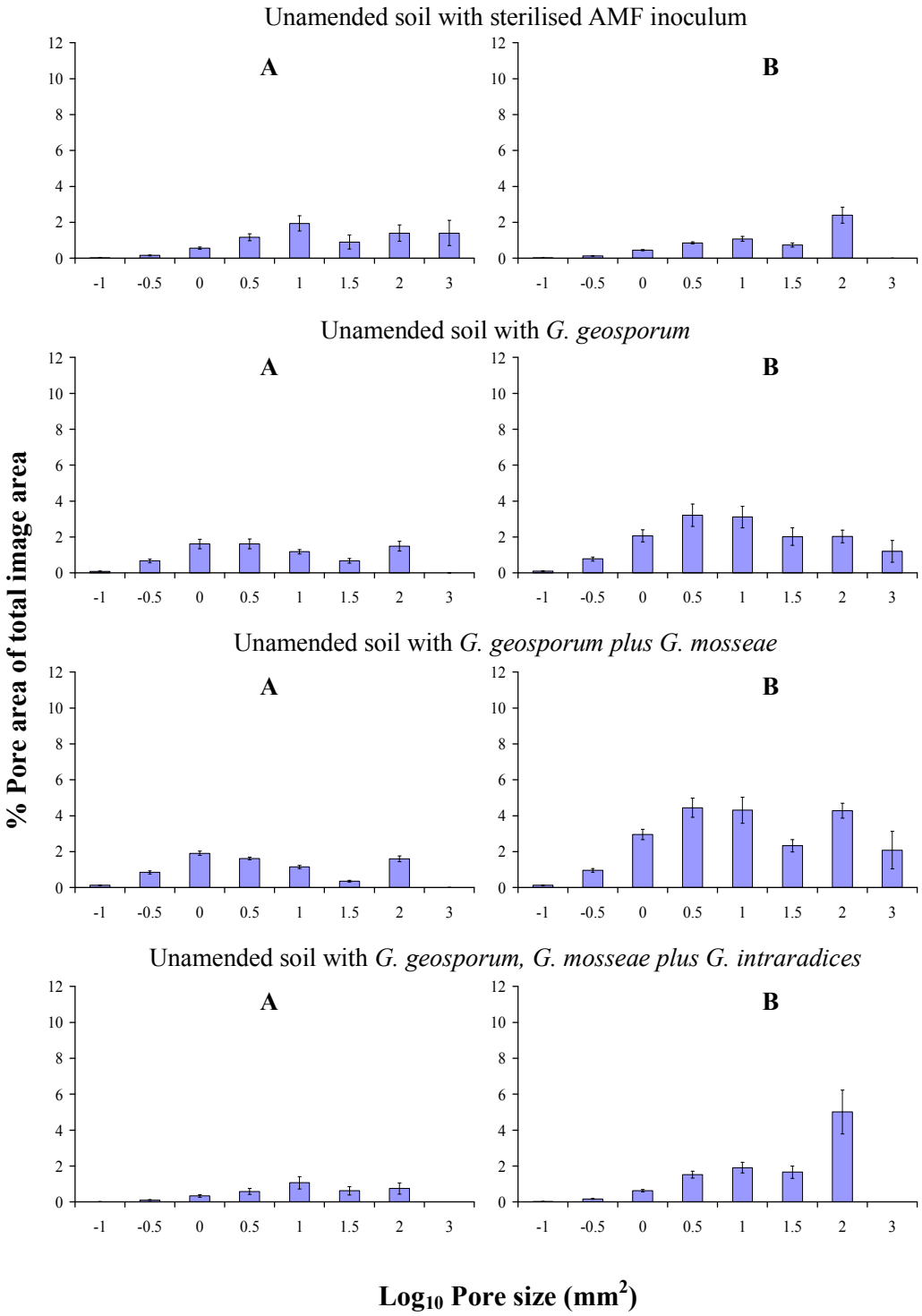


Figure 5.36: ***Continued***

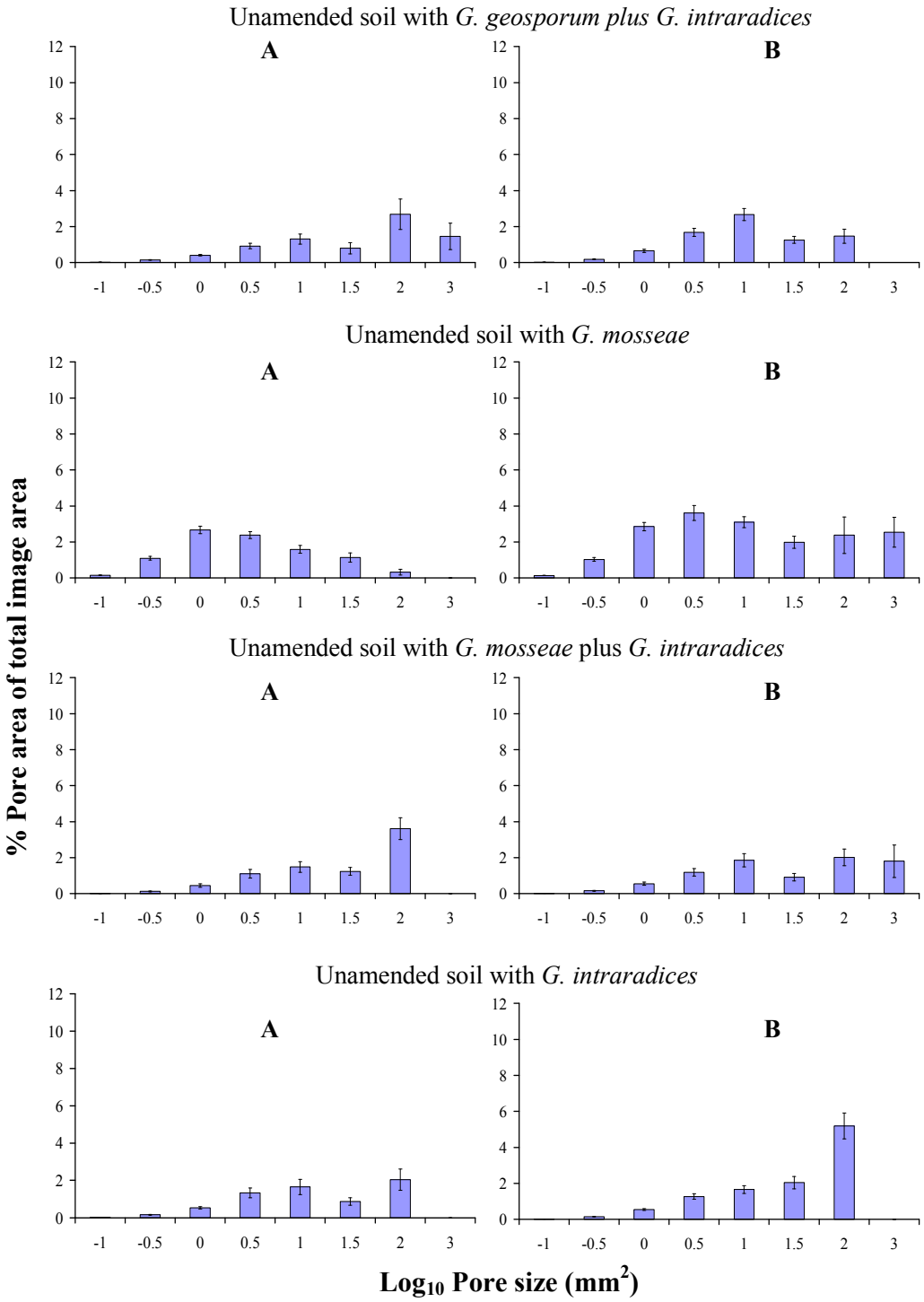


Figure 5.36: Pore size distributions from A) unplanted and B) planted sides of the macrocosms after 7 weeks of incubation.
Data are means ± standard errors.

5.3.14 PORE SPHERICITY

Pores at the start of the investigation were more circular (0.341) than those at the end of the experiment (0.308), which was expected since sphericity is a function of pore size, with a smaller pore size generally resulting in a more rounded pore (time effect; Table 5.8). At the start of the investigation, sphericity was highest within the slurry-amended compared to the unamended soil (0.343 and 0.338 respectively); by the end of the investigation pore sphericity was highest in the unamended compared to the amended macrocosms (0.313 and 0.303) despite a general decline in sphericity values overall (dilution x time interaction; Table 5.8). Furthermore at the start of the investigation there was no significant difference in sphericity between the unplanted (0.341) and planted (0.340) (time x location interaction; Table 5.8). However, at the end of the experiment, pore sphericity was highest within the planted side of the macrocosm (0.312) compared to the unplanted side (0.304). Whilst differences in sphericity are statistically significant, they are nevertheless very small.

Pores within the 3-species AMF mix were significantly more elongated than those within other treatments (AMF species as single factor; Table 5.8). Pores were more rounded within the treatments containing *G. geosporum* and *G. mosseae* individually and also in combination. Pores within the control (i.e. macrocosm with sterile AMF inoculum) had a sphericity value higher than that of the 3-species mix, similar to that of the *G. geosporum* plus *G. intraradices* mix and *G. mosseae* plus *G. intraradices* mix, but was significantly lower than *G. intraradices* individually. Patterns in sphericity, did not directly reflect a relationship with mean pore size, particularly within the treatments containing *G. geosporum* and *G. intraradices* individually, the 3-species mix and the

control, suggesting plant roots and/or mycorrhizal fungal species influenced pore shape.

Sphericity was affected by the number of AMF species present at the final harvest (Figure 5.37).

Table 5.8: Results from repeated measures ANOVA for pore sphericity.

Source of variation	DF	F	P
AMF species	7	11.44	< 0.001
Slurry amendment x AMF species	7	6.61	< 0.001
AMF species x split macrocosm side	7	2.53	0.017
Slurry amendment x AMF species x split macrocosm side	7	7.14	< 0.001
Residual	*155*		
Time	1	302.42	< 0.001
Time x slurry amendment	1	16.12	< 0.001
Time x AMF species	7	7.06	< 0.001
Time x split macrocosm side	1	6.61	0.011
Time x slurry amendment x AMF species	7	7.44	< 0.001
Time x AMF species x split macrocosm side	7	2.47	0.020
Time x slurry amendment x AMF species x split macrocosm side	7	8.35	< 0.001
Residual	*160*		

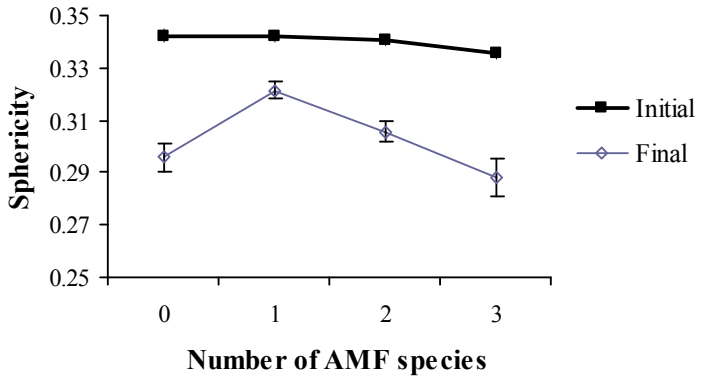


Figure 5.37: Effect of number of AMF species within the macrocosms on the sphericity value of pores determined from image analysis.
Data are means ($log_{10}+1$) ± standard error. ANOVA of data taken from the final harvest shows a significant difference ($F_{3,183} = 9.42$, $P < 0.001$).

The outcomes of treatment are complicated as evidenced by the time x slurry x AMF species x location interaction (Table 5.8; Figure 5.38). Generally, sphericity within the split macrocosms was highest within the initial time zero (seedling stage) scan in comparison to the final scan, despite the values being relatively low suggesting the dominance of more elongated pores. At the initial scanning period there was some variation between treatments, with macrocosms containing *G. intraradices* individually and *G. geosporum* plus *G. intraradices* having a higher sphericity within the unplanted side of the column in unamended soil, sphericity was higher in the planted side of the macrocosm within the treatments containing *G. geosporum* individually, when mixed with *G. intraradices* and also within the *G. mosseae* plus *G. intraradices* mix. These responses may be regarded as trivial and not unexpected at that stage in the experiment.

After 7 weeks, treatment responses were more apparent. Within the amended soils, sphericity was lowest in the unplanted side of the macrocosms containing *G. intraradices* individually and when mixed with *G. mosseae* and also within the *G. geosporum* plus *G. mosseae* combination. However, within the non-mycorrhizal columns the pattern was the opposite, with pore sphericity highest in the unplanted side of the macrocosm. Within the unamended macrocosms, sphericity was lower in the unplanted side within treatments containing sterile AMF inoculum, *G. geosporum* plus *G. intraradices* and also the 3-species mix. The opposite was true however for *G. intraradices*, where sphericity was highest within the unplanted side of the macrocosm. Interpretation of these data should be carried out with caution given the small values involved.

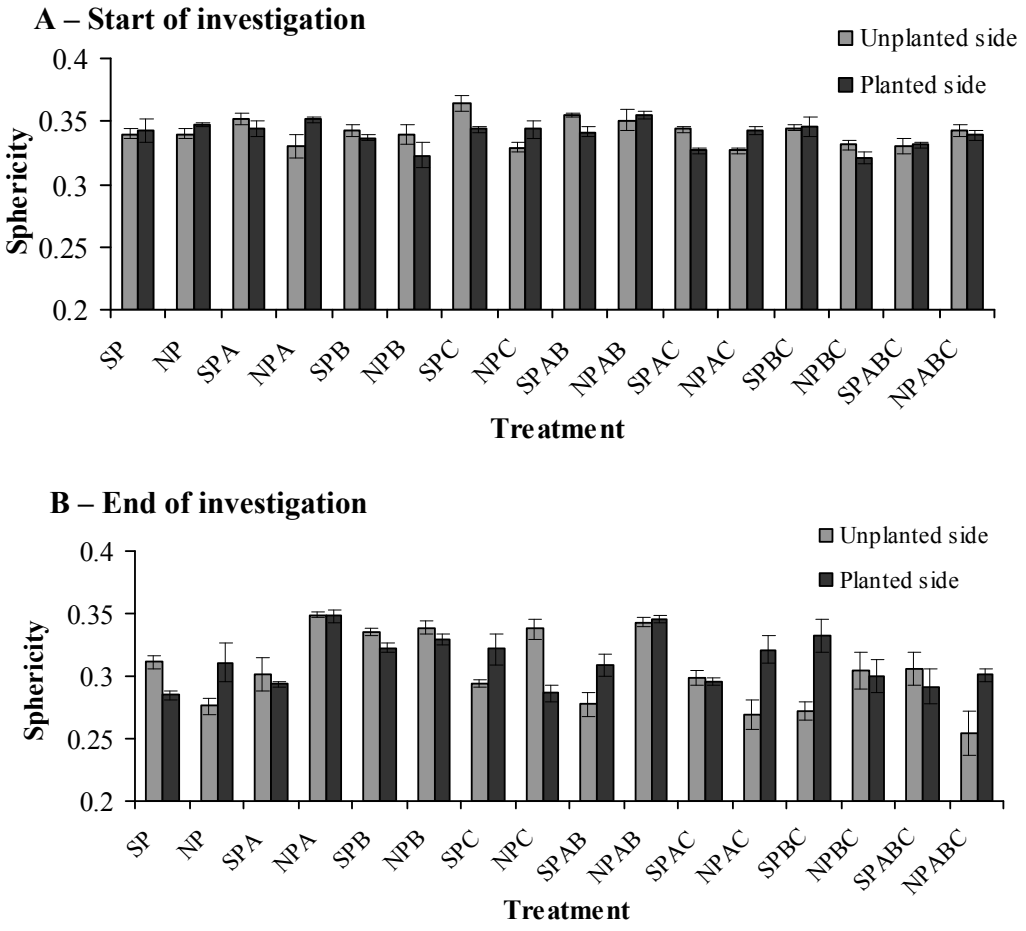


Figure 5.38: Pore sphericity determined from analysis of X-ray CT images at A) the start and B) end of the investigation ($F_{7,160}$ = 8.35, P < 0.001).
Data are means (log_{10}+1) ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

5.3.15 NEAREST NEIGHBOUR DISTANCE

Macrocosms containing all three AMF species (1.12 mm) and the *G. geosporum* plus *G. intraradices* mix (1.11 mm) had significantly greater distance between pores within the macrocosm ($F_{7,155}$ = 14.50, P < 0.001). The smallest distance between pore spaces was observed within the macrocosm containing *G. mosseae* individually (0.95 mm).

Furthermore, Figure 5.39 illustrates that with increasing mycorrhizal species number, distance between pore spaces tends to increase.

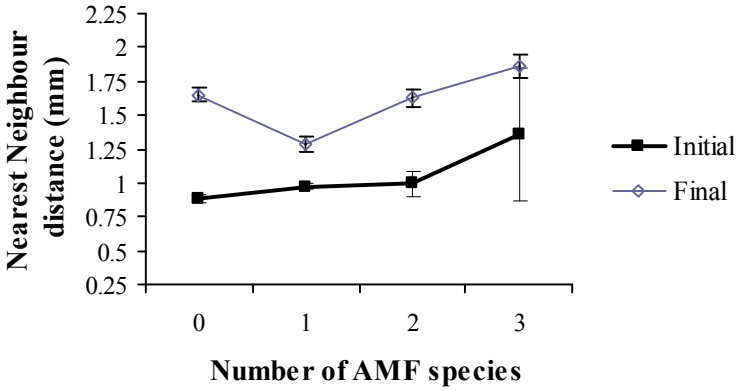


Figure 5.39: Effect of number of AMF species within the macrocosms on nearest neighbour distance determined from image analysis.
Data are means ± standard error. ANOVA of data taken from the final harvest shows a significant difference ($F_{3,183}$ = 11.895, P < 0.001).

Slurry amendment of macrocosms containing *G. geosporum* individually and when combined with *G. mosseae* resulted in an increase in the nearest neighbour diameter between pores. In the remaining two macrocosms containing *G. geosporum* (i.e. the 3-species mix and when combined with *G. intraradices*) a similar (but not significant) increase in nearest neighbour diameter was observed within the slurry amended macrocosms. In the remaining macrocosms there was a decrease in nearest neighbour pore distance with the addition of the soil slurry, however this was only significant with the macrocosm containing *G. mosseae* individually (slurry x AMF interaction, $F_{7,155}$ = 4.81, P < 0.001).

The distance between pores increased from 0.97 mm to 1.16 mm ($F_{1,160}$ = 325.91, P < 0.001) from the starting point (seedling emergence) to the final 7 week harvest. The one exception was the *G. mosseae* treatment which showed a reduction in nearest

neighbour distance over time (AMF x time interaction, $F=_{7,160}$ = 17.13, P < 0.001; Figure 5.40).

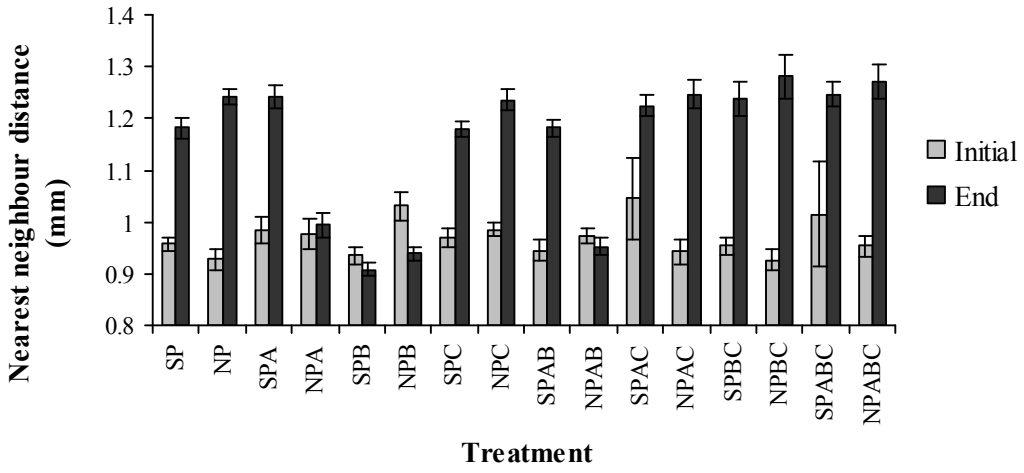


Figure 5.40: Average nearest neighbour distance determined from analysis of X-ray CT images at the start and end of the investigation for each macrocosm

Data are means ± standard error. (Treatment codes: SP represents soil slurry amendment, NP represents no slurry amendment. Codes containing A, B and C relate to AMF species with A = G. geosporum*; B* = G. mosseae *and C* = G. intraradices).

5.3.16 LINKING SOIL PHYSICAL MEASUREMENTS WITH MICROBIAL MEASUREMENTS.

A significant relationship was observed, between Simpson's bacterial diversity index and (i) pore sphericity and (ii) the coefficient of uniformity calculated from aggregate size distributions from the **unplanted** side of the macrocosm. Figure 5.41A and B show that as bacterial species diversity declines, both pore sphericity and aggregate size increase. Further correlations were observed within the data from the unplanted side of the macrocosm (Table 5.9).

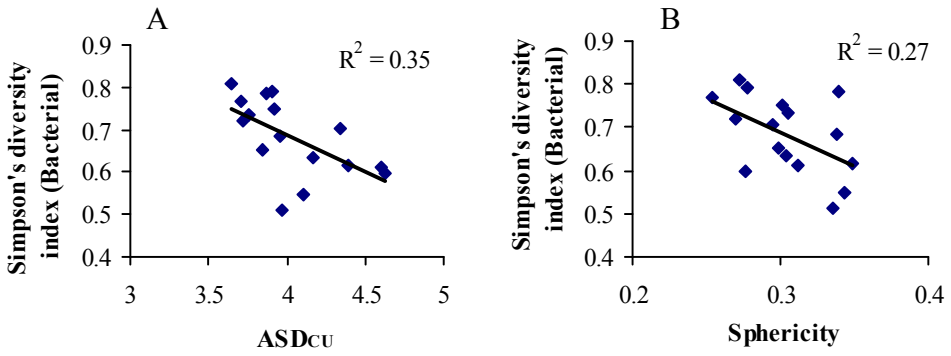


Figure 5.41: Regression between bacterial species diversity and A) the coefficient of uniformity value for aggregate size distribution data (P = 0.016) and B) pore sphericity (P = 0.04). Data for AMF mixes and slurry amendment are combined.

Table 5.9: Selected correlation matrix of significant (P < 0.05) relationships on the unplanted side of the macrocosm. Data for AMF mixes and slurry amendment are combined.

NB: Shaded boxes are not significant relationships. Critical value 0.468.

	ASDcu	Pore sphericity	Fungal species richness	Bacterial species richness	Organic Matter
Aggregate stability	+ 0.4822				
Simpson's diversity index (Fungal)	0.2939	0.0366	+ 0.688		
Simpson's diversity index (Bacterial)	- -0.6135	- -0.525	- -0.4816	+ 0.5077	
Soil Biomass	-0.0982	0.2022	-0.2668	- 0.0811	+ 0.4895
Log mean pore area	0.1657	- 0.4786	-0.2852	0.2207	-0.1939

Structural measurements correlated strongly with plant and microbial analyses particularly when assessing measurements made on the **planted** side of the macrocosms. A strong relationship was observed between fungal species richness and the coefficient of uniformity of aggregate size distribution data (Figure 5.42), in addition to a significant correlation ($P < 0.05$) between fungal species diversity with

ASD_{CU} (data not shown). The results show as fungal richness decreases, the coefficient of uniformity value increases (suggesting a greater number of larger soil aggregates). In addition, a strong relationship between fungal diversity and aggregate stability was evident. As fungal species diversity decreases aggregate stability increases (Figure 5.43). There was a strong correlation ($P < 0.05$) between fungal species richness and aggregate stability. These results therefore highlight the importance of fungal diversity within the planted side of the macrocosm and bacterial diversity within the unplanted side on aggregate size distributions.

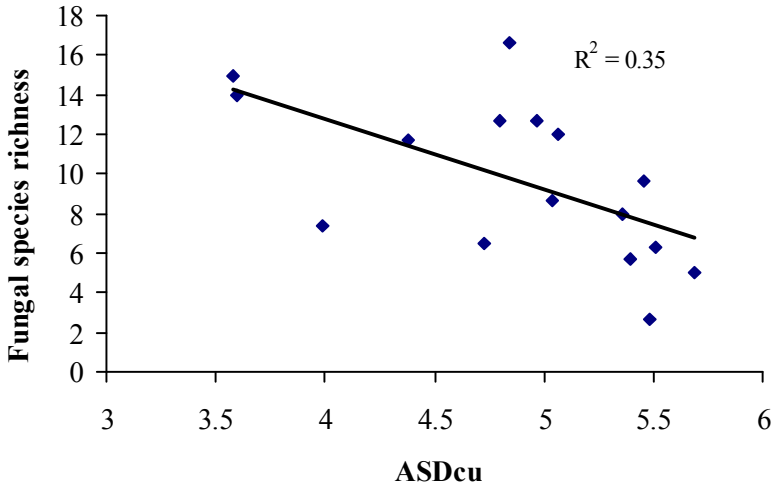


Figure 5.42: Regression between fungal species richness and the coefficient of uniformity of aggregate size distribution data from the planted side of the macrocosm. Data for AMF mixes and slurry amendment are combined, ($P < 0.016$).

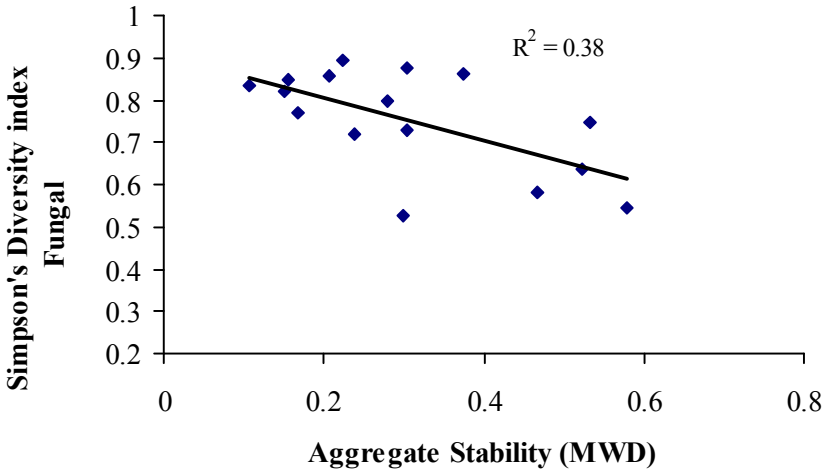


Figure 5.43: Regression between Simpson's diversity index for fungi and aggregate stability for each fungal species mix and in planted macrocosms with and without soil slurry added, (P < 0.01).

5.4 DISCUSSION

5.4.1 IMPACT OF SOIL SLURRY ADDITION

No differences in bacterial diversity (Simpson's index) between the slurry amended and unamended soils were observed but interestingly, effects of slurry amendment on fungal diversity depended on the AMF species present. Nevertheless, amendment generally increased soil fungal diversity in mycorrhizal columns relative to the non-mycorrhizal treatments. This may be due to direct synergistic interactions, or to indirect effects mediated by mycorrhizal-induced alterations in quality or quantity of root exudates (Bansal and Mukerji, 1994; Filion *et al*., 1999; Marschner and Baumann, 2003; Vierheilig, Lerat and Piché, 2003). It is worth noting that the bulk soil was analysed here and further effects would be expected within the mycorrhizosphere (Johansson, Paul and Finlay, 2004), although Marschner and Baumann (2003) reported

mycorrhizal-induced alterations in bacterial communities in non-rhizosphere soil. Mycorrhizal colonisation of *P. lanceolata* roots was unaffected by soil amendment when a single species of AM fungus was present, but negatively affected when in combination. It is known that AM fungi are influenced by rhizobacteria (Fitter and Garbaye, 1994) and that plant host can play a role in regulating AMF colonisation (Eom, Hartnett and Wilson, 2000). The findings of this investigation suggest that either factor, and/or direct AMF competition regulate root colonisation.

Although soil bacterial diversity appeared unchanged following slurry amendment, there were differences in the T-RFLP profiles suggesting that community composition was altered. For bacteria, slurry amendment influenced PC1 within the unplanted side of the macrocosms, and PC3 within the planted side, with soils from amended and unamended macrocosms each containing unique fragments. Unique fungal T-RFs were observed in the planted side of the columns. Thus soil amendment influenced microbial community composition within the macrocosms, but only with regard to 2-5 T-RFs.

The similarity in bulk soil microbial profiles is interesting considering one set of columns was inoculated with soil slurry and the other with sterile water. It would be expected, that over the course of the experiment the unamended soils would develop a significant microbial community resulting from aerial introductions, bacteria within the AMF inoculum, non-sterile plant seed, and development within the soil if not fully sterilised at the start. Nevertheless, greater differences should be expected between the two treatments than were detected by T-RFLP analysis. Although the number of T-RFs detected were broadly in the region of others (e.g. Kennedy *et al.*, 2004), the method is

limited because many species share the same fragment length (Dahllöf, 2002). Therefore, rather than one fragment representing one species, it is more likely here to represent a genus or other grouping, such as the bacterium identified in this study that belonged to the *Burkholderia* genus, a bacterium associated within AMF spores (Bianciotto *et al*., 1996; Andrade *et al*., 1997). Despite these criticisms of the method, T-RFLP analysis allows for differentiation of relative diversity.

Nevertheless, slurry amendment led to an increase in biomass-C in unplanted sides of the columns and visual assessments of hyphal presence verified that amendment introduced fungi to the system. This is reflected by measurements of aggregate stability, total porosity and mean pore size which all increased with the addition of soil slurry. These results highlight that despite the limited evidence in a change of diversity, increased microbial biomass (and even total bacteria numbers (Wertz *et al*., 2006)) have a significant impact on soil structure (Drury, Stone and Findlay, 1991; Edgerton *et al*., 1995).

Griffiths *et al. (*2001) recommended that after inoculation using a soil dilution, soil should be incubated at 15 ^{o}C, for 9 months, with soil mixed every two weeks to allow an even development of microbial communities. Since the columns here were inoculated prior to seed germination (at the start of the investigation), no time was given to allow the soil communities to develop and reach evenness before the start of the investigation. This was essential if soil structural development was to be measured over time. Nevertheless, this would not explain the apparent lack of difference in species richness based on T-RFLP data.

5.4.2 *IMPACT OF LOCATION WITHIN THE SOIL MACROCOSM*

Location within the soil macrocosm had a significant impact on both biological and soil structural properties. With regard to both bacteria and fungi, presence within the planted side of the macrocosm, resulted in an increase in richness and diversity. This is due to the biochemical impacts that plants and mycorrhizal colonisation have on the planted soil compared to that of the unplanted where mycorrhizal hyphae (but not roots) had penetrated. Plant roots and mycorrhizal fungi release polysaccharides and other exudates that act as substrates to microbes, hence allowing an increase in species richness and diversity. In addition, soil biomass was also noticeably higher within the planted side of the macrocosm due to stimulation by the roots (Denef *et al.,* 2002), that further synthesise polymers into binding agents (Jastrow, Miller and Lussenhop, 1998).

The exudates released by roots and mycorrhizal fungi in addition to acting as microbial substrates, also act as binding agents to soil particles (Tisdall and Oades, 1982) resulting in aggregation in addition to increased stability. Indeed, increased aggregate size and stability within the planted side of the macrocosms was observed in this investigation. The impact of root exudates appeared to be far more important than that of mycorrhizal exudates, whose presence within the unplanted side of the macrocosms had little impact on aggregates compared to that of the roots within the planted side. Hallett *et al. (*2009) who also used split macrocosms, discussed that wet-dry cycles mediated by plants, lead to an increase in water stable aggregates, since drying causes the cohesion of soil particles, in addition to an increase in microbial respiration (Magid *et al*., 1999; Cosentino, Chenu and Le Bissonnias, 2006). However, previous studies by Denef *et al. (*2002) suggested that after short growing periods (i.e. 42 days) root growth

may stimulate macro-aggregate breakdown more than increasing their stability, since there would be insufficient production of stabilising agents to increase stable macro-aggregation. Furthermore root morphology is also vital in the distribution of organic matter that stabilises the soil. Since *P. lanceolata* roots are fine in nature, Degens (1997) suggests that organic material inputs would be more evenly distributed compared to that of coarse root systems.

In the current study, other structural analyses such as total porosity and mean pore area were all higher within the planted side of the macrocosms. The increase in total porosity and mean pore size would be expected since the biophysical action of roots results in the movement of soil particles, binding them together creating additional pore space and also pore enlargement. Moreover the presence of root material within the soil is not always differentiated by image analysis, due to the poor contrast between air filled pore space and root material within the bulk soil, thus overestimation of total porosity and mean pore area within the planted side of the macrocosms may have also occurred.

Such development of soil structure within the planted side of the macrocosms highlights that the driving force behind aggregate formation, in addition to the development of porous spaces within the soil was due to root activity, both direct and indirect (mediated *via* exudates and increased microbial activity). This is in agreement with previous work by Hallett *et al. (*2009) whose work with split column systems also found roots to be the main emphasis on formation and stabilisation of soil structure.

5.4.3 *IMPACT OF AM FUNGAL SPECIES OF SOIL STRUCTURE AND SOIL MICROBES*

Unfortunately the inoculum used in this experiment was not as effective as that previously used in chapter 4, with colonisation rates of 0-34 % observed after 7 weeks. This may have been due to the ineffectiveness of the chlorazol black E stain used in the assessment, since Klironomos, McCune and Moutoglis (2004) highlighted that some AMF species are not detected as well using some staining methods. Furthermore, Endlweber and Scheu (2006) highlighted that after inoculating sterilised soil, re-colonisation of AMF is hampered by higher nutrient contents such as that of nitrogen as measured by Blanke *et al. (*2005). Nevertheless, AMF colonisation was significantly higher in the AMF species mix of *G. geosporum* plus *G. mosseae*, in addition to the three species mix. Relatively high colonisation rates were observed in macrocosms containing *G. geosporum* individually and in combination with *G. intraradices*, with lowest rates observed in treatments containing *G. mosseae* and *G. intraradices* individually and when mixed. Such variation in colonisation rates associated with different AMF species has been previous observed by van der Heijden *et al. (*1998a). Moreover Garbaye (1994) proposed that some rhizobacteria increased the ability of a root to establish symbiotic relationships with mycorrhizal fungi, a process termed as 'mycorrhization help bacteria'. He suggested that such helper bacteria aided stimulation of root development and enhanced susceptibility and recognition of roots to mycorrhizal colonisation, a process that may well have taken place within this investigation with some mycorrhizal species. Andrade *et al. (*1997) also highlighted that different bacterial populations establish themselves under the influence of different AMF species and hypothetically influence the number of helper bacteria present within the rhizosphere and hydrosphere. In addition, the interaction of plant growth-promoting

rhizobacteria (PGPR) (bacteria which increase plant yield through numerous mechanisms as described in the review of Artursson, Finlay and Jansson, (2006)) can also behave similar to 'mycorrhization helper bacteria' (Garbaye, 1994) by optimising the formation and functioning of mycorrhizal symbiosis (Azcón, 1987; Linderman, 1997 and Artursson, Finlay and Jansson, 2006).

Dry root biomass was influenced by fungal species and it can be clearly seen that in the macrocosms with low colonisation rates such as those of *G. mosseae* plus *G. intraradices*, root biomass was highest, whereas within the macrocosm of *G. geosporum* plus *G. intraradices* where colonisation rate was among the highest, root biomass was the lowest. This reflects the results in section 4.3.2 where root biomass was lowest in macrocosms containing mycorrhizal fungi. It must be noted different AMF species not only have different colonisation rates (Hart and Reader, 2002), growth rates and methods of colonisation within individual plants (Hart, Reader and Klironomos 2001; Hart and Reader, 2002), but interact differently with host plant (Klironomos, 2003) leading to a differing need for plant carbon (Staddon, 1998; Saikkonen *et al*., 1999). However AMF species not only control the host plant and its productivity, but it has also been found that AMF morphology is dependent on the plant type (Smith and Smith 1997; Cavagnaro *et al*., 2001).

There was a trend towards increased root (and shoot) biomass with increasing number of AMF species within the macrocosms, similar to that observed by others (e.g. van der Heijden *et al.,* 1998b; Klironomos, McCune and Moutoglis, 2004; van der Heijden *et al.*, 2006). Furthermore, increasing number of AMF species led to decreased ASD_{CU},

total porosity and pore sphericity due to increased quantity of root material, with nearest neighbouring pore distance increasing because of compaction of the rhizospheric soil by root activity. The AMF-induced reduction in ASD_{CU} is interesting because this parameter was greater in planted than in unplanted soil. Increasing the number of AMF species clearly had a negative affect, either in terms of increasing root growth over a threshold value, or by hyphal action (contradicting hypothesis four, section 1.5). AMF hyphae are normally associated with increased aggregation by physical binding and production of glomalin (Tisdall and Oades, 1982; Bearden and Petersen, 2000; Rillig, Wright and Eviner, 2002; Piotrowski *et al.*, 2004).

In terms of other soil biological measurements, AMF species mix had a significant effect on soil biomass. Macrocosms with high colonisation levels tended to have increased soil biomass. Also, bacterial species diversity was influenced by the number of AMF fungi present within the soil, increasing from none to two AMF species, before declining when all three AMF species were present. Furthermore fungal species richness increased on the planted side of the macrocosm with increasing number of AMF species as would be expected, but the reverse effects on species richness were observed on the unplanted side. Fungal richness and diversity were influenced by AMF species combination in addition to the number of species. Decreasing fungal richness on the unplanted sides of the columns is interesting and is likely to reflect changes in saprophytic populations. Fracchia *et al.* (1998) demonstrated that *G. mosseae* reacts to a range of saprophytes antagonistically, synergistically or neutrally and it is likely that any of the *Glomus* species used here could behave in a similar way. Therefore, extraradical growth through the central mesh may have affected other microbes present

on the unplanted side of the columns. The results in general are in agreement with the widely acknowledged belief that AMF influence microbial populations (Andrade *et al*., 1998; Artursson and Jansson, 2003) and that different AMF species have different impacts on soil microbial composition (Rillig *et al.,* 2006). The effect of AMF species on soil bacterial abundance and activity was further highlighted by Filion, St-Arnaud and Fortin (1999) with further suggestions by Ravnskov, Nybroe and Jakobsen (1999) that changes in bacterial and fungal composition within soil containing AMF may be due to the release of bacteriostatic or fungistatic agents from the hyphae. Schreiner *et al. (*1997) also illustrated differences in Gram negative and Gram positive bacteria with different AMF species colonising soybean plants.

Soil structural properties were influenced by AM fungal species with significant effects on all pore characteristics. However, AMF did not effect other soil structural properties relative to the planted non-mycorrhizal treatments which is contrary to hypothesis three (section 1.5). Mean pore size was lowest within the macrocosms containing *G. mosseae* which is a reflection of the low colonisation rates observed. No significant effect of AMF species on aggregate stability was observed perhaps due to the shortness of the investigation despite work from Schreiner *et al. (*1997) and Piotrowski *et al. (*2004) highlighting that different AMF species lead to different levels of water stabile aggregates. However, Piotrowski *et al. (*2004) found the lowest percentages of water stable aggregates in the presence of *Glomus* species in *P. lanceolata,* thus suggesting the AMF genus selected for this investigation generally had a small effect on the stability of aggregates.

5.4.4 INTERACTIONS BETWEEN MICROBIAL AND SOIL PHYSICAL PROPERTIES

The results suggest that aggregate stability and development increase when species richness and diversity is lower, hence suggesting that as interactions between different fungal species decline or are reduced, the impact of fungi on aggregate development and stability increases. The soil biota (particularly the presence of AMF) has been found to alter plant exudation (Graham, Leonard and Menge, 1981) which could ultimately affect aggregation. With regard to the effect soil bacteria can indirectly have on soil structure, some strains have been found to have stimulatory or inhibitory effects on mycorrhizal colonisation (Fitter and Garbaye, 1994), thus ultimately influencing the impact mycorrhizal hyphae have on soil structure such as the release of glomalin and related soil proteins (GRSP) that are known to improve soil aggregation and stability to a greater extent than AM hyphal activity *per se* (Rillig, Wright and Eviner, 2002).

5.5 CONCLUSION / SUMMARY

- Within the planted side of the macrocosm, bacterial species diversity and species richness of bacteria and fungi were higher. This was most likely due to the biochemical impact roots have by providing polysaccharides and other substrates for biota to utilise.

- The presence of roots within the planted side of the macrocosms appear to be the driving force behind soil structure development, particularly aggregate stabilisation and the increase in pores.

- Mycorrhizal colonisation of *P. lanceolata* varied according to AMF species mix applied to the soil macrocosms. Generally colonisation rates were low throughout the investigation, either due to changes in soil nutrient content after sterilisation limiting mycorrhizal colonisation, ineffective inocula or due to limitations associated with the staining method used.

- The 3-species mix of AMF species increased shoot and root biomass relative to when the species were found individually and in pairs.

- Bacterial community composition (assessed by T-RFLP) and soil biomass were also influenced by mycorrhizal species mix, with soil biomass generally highest in macrocosms with high rates of AMF colonisation.

- Soil properties were influenced by mycorrhizal species mix, with mean pore size lowest in the macrocosms containing *G. mosseae*, whose colonisation rate were lowest. Generally however soil structure development was greatest under the influence of plant roots.

- Bacterial species richness influenced aggregate size distribution within the unplanted macrocosms, with an increase in larger aggregates associated within soil containing low bacterial richness. Fungal diversity and richness had a significant and similar relationship on soil structural measurement of aggregate stability and ASD_{CU}. Hence this investigation suggests that as fungal diversity decreases, aggregate stability increases and as fungal and bacterial species diversity decline, aggregates become larger. These results also highlight that

within the planted side of the macrocosms (where pores were larger) it appears as if fungi had more impact on soil structure (after that of roots), whereas within the unplanted side of the macrocosms bacteria had more impact.

- A general conclusion therefore is that changes in soil structure observed here was predominantly due to direct effects on roots and their biochemical release, rather than to the mycorrhizal fungi.

6 GENERAL DISCUSSION

6.1 APPROACH AND GENERAL FINDINGS

The main objective of this work was to evaluate the role of soil biodiversity on the development of soil structure. Different experimental approaches were used throughout this study with macrocosms being most widely employed, either whole (Chapter 4) or split, in order to assess the impact plant roots and mycorrhizal hyphae have separately on soil structure (Chapter 5). Smaller microcosms were used within the first (trial) experiment (Chapter 3) for micro-scale assessment of changes taking place in soil structure. Different background microbial communities were applied to different experiments using a modification of the dilution technique described by Griffiths *et al.* (2001), with soil conditions varying from bare soil (Chapter 3) to planted ± mycorrhizal fungi (Chapter 4). Mycorrhizal diversity ranged from a complete five species mix, where the effect of mycorrhizal and non-mycorrhizal roots on soil structure was assessed (Chapter 4), to variations in species mixtures and individual species to assess the effect of mycorrhizal fungi species diversity on soil structure (Chapter 5). A summary of the key findings follows:

- Soil texture influenced soil structure and subsequently microbial communities within the soil.
- Increased levels of organic matter increased aggregate stability.
- Soil structure development measured through porosity, took place within columns after an initial settling period.

- Soil microbial communities influenced soil structure, even though these observed changes were smaller and slower than those observed under planted systems.
- As soil microbial numbers increase, soil structural properties such as total porosity and mean pore size increase.
- Mycorrhizal roots influenced aggregate stability more than roots or mycorrhizal fungi individually. Furthermore the effects of roots individually were greater than those of mycorrhizal hyphae when alone in bulk soil.
- The presence of mycorrhizal fungi and combination(s) of AMF species influenced the bacterial community.

6.1.1 Influence of Soil Texture on Soil Structure and Microbial Communities

Chapter 3 highlighted that soil structural properties and the development of soil structure (measured through the assessment of aggregates and the associated pore characteristics) were significantly modified by microbial communities within the microcosms, with the effect dependant on the soil texture over the course of the investigation. Loamy sand had the highest porosity and mean pore size compared to the other soil textures, namely sandy loam and clay loam. This is because the loamy sand soil contains a relatively high percentage of sand, i.e. large particle sizes resulting in larger pore space than that of clay soils, which have fine particles, that can lead to clogging of pores and pore throats (Mooney, 2002). However, this may also partly be a function of the overall resolution, since the bigger pores will be more readily

determined by image analysis, compared to the smaller pores of the clay loam, which could be incorrectly assumed to be noise thus underestimating the porosity within this soil type. Soil texture also had a significant effect on culturable microbial communities within the microcosms with clay loam soil having the highest culturable fungal numbers (and second highest bacterial numbers) due to the higher organic matter content (Barros, Feijoo and Balsa, 1997).

It is widely acknowledged that for a soil to maintain a good soil structure, aggregates need to be stable. Chapter 4 highlighted the importance of soil organic matter on aggregate stability, with soils that had a low microbial biomass (hence lower demand for organic substrates resulting in a higher soil organic matter content) having aggregates more stable than those in treatments containing a higher microbial biomass and lower organic matter content. This agrees with the general consensus that soil organic matter is important in aggregate formation and particularly stabilisation in micro-aggregates leading to the formation of stable macro-aggregates (Six *et al.*, 2004).

6.1.2 Effect of Time on Soil Structure

The development of soil structure overtime, varied depending on the soil environment i.e. soil texture and presence of plants and AMF. In Chapter 3 soil structure development was observed in microcosms containing three different soil textures (sandy loam, clay loam and loamy sand). Soil structure within these bare soil microcosms noticeably improved over time after an initial soil settling period (i.e. where soil within the microcosm compacted and porosity reduced, such as that observed by Leij, Ghezzehei and Or, (2002) after tillage). Total porosity and mean pore

size almost regained or increased compared to the values observed initially. Overall Chapter 3 illustrated that soil structural re-development took place over a nine month period in spite of the time-related reduction in microbial activity and number of colony forming units. Settling of the soil within macrocosms was also observed in Chapter 4 however despite this, aggregate size distribution, porosity and mean pore size increased (with a decline in distance between individual pore spaces also observed) in the fifth month suggesting signs of soil structure development over a 2 month period. This improvement in soil structure did not continue into the seventh month due to soil macrocosms becoming root bound causing the roots to compact the available pore space as they grew and spread throughout the soil (Brund *et al.,* 1996) resulting in individual pores becoming located closer together than previous.

Development in soil structure at the macroscale was determined through assessment of porosity and mean pore area, with these data being the first in the research area. Previous assessment at the aggregate scale, highlighted individual aggregate turnover rates ranging from 4-88 days (Plante, Feng and McGill, 2000; De Gryze *et al.,* 2005; De Gryze, Six and Merckx, 2006). At an individual aggregate scale within this study, Chapter 4 highlighted an increase in aggregate stability over a 60 day period (from the first to third month harvest) and an increase in aggregate water repellency over a 120 day period (from the first to fifth month harvest). Such time periods for aggregate stabilisation and repellency appear to be comparable to those of aggregate turnover rate (Plante, Feng and McGill 2000; De Gryze *et al.,* 2005; De Gryze, Six and Merckx, 2006). Despite development of stable aggregates over a 60 day period, development of porosity (i.e. compared to that initially introduced) took place over much longer time

periods, with the only increase in porosity observed after 15 months (in the bare soil treatments of Chapter 3). This was much slower than that observed by Feeney *et al. (*2006a) who illustrated increases (between 3-7 %) in soil porosity after a 30 day period within bulk and rhizospheric soil. In spite of this, porosity changes generally take place over much longer periods than aggregate turn over, particularly at a field scale, where soil structure development can take many months to years (Elliott and Coleman, 1988; Boersma and Kooistra, 1994).

6.1.3 Effect of Plants, Roots and AMF on Microbial Communities and Soil Structure

In Chapter 4 soil biomass, total substrate utilisation from Biolog microtitre plates and fungal species richness were all lowest within the bare soil environment, highlighting the importance of microbial communities associated with root and mycorrhizal exudate release within the soil (Fitter and Garbaye, 1994; Jaeger *et al*., 1999; Walker *et al.,* 2003; Nappipieri *et al*., 2008). Chapter 5 also highlighted that microbial species richness and diversity were all higher within soil located within the planted half of the split macrocosms. T-RFLP profiles from Chapter 4 further showed that the soil environment influences the microbial community composition, with particular differences observed between the bare soil and planted macrocosms with the presence and absence of different fragments determined from principal component analysis (PCA). Differences in bacterial community composition between bare soil and rhizospheric soil have been observed previously by Baudoin, Benizri and Guckert (2002); Marschner and Baumann (2003) and Remenant, Grundmann and Jocteur-Monrozier, (2009). Furthermore, the effect of plants and their roots on microbial

populations was further highlighted in Chapters 4 and 5, with a close positive relationship between microbial and root biomass. Such findings were similar to those reported in previous research by Lynch and Whipps (1990) and Bardgett (2005).

Chapter 4 illustrated that AMF increased aggregate stability, with the impact of roots and mycorrhizal fungi on aggregate stability being greater than that of roots alone. van der Heijden *et al*. (2006) outlined such a relationship, with mycorrhizal roots increasing aggregate stability and even to some extent water percolation through a soil, compared to a treatment with uncolonised roots. This finding was further tested in Chapter 5, which illustrated that within the planted side of the macrocosms (that also contained mycorrhizal fungi) an increase in aggregate stability was observed. Results from Chapter 5 highlighted that soil with roots and mycorrhizal hyphae influenced aggregate stability more than the impact of hyphae alone, similar to the findings of Hallett *et al.* (2009). Further investigation of mycorrhizal fungi was undertaken in Chapter 5 with the assessment of the effect mycorrhizal fungal diversity had on soil structure. Different AMF species had different colonisation rates and hence differential effects on root biomass, with root biomass lowest in macrocosms containing the highest percentage root length colonised. However, an interesting pattern was observed with root (and shoot) biomass increasing as the number of AMF species within the mixture increased. A negative relationship of AMF species number with soil structure was observed in chapter 5, but instead highlighted the importance of roots rather than AMF have on soil structure development. Furthermore different mycorrhizal species also influenced microbial biomass (in addition to bacterial and fungal community composition) with the highest mycorrhizal colonisation rate associated with the highest

microbial biomass. T-RFLP profiles also illustrated that the presence of *G. geosporum* regardless of the other additional species (probably identified as fragment 260 bp), resulted in a similar 'background' fungal community structure compared to the other mycorrhizal species investigated. Moreover Chapter 5 illustrated that mean pore size was lowest within the macrocosms containing *G. mosseae* due to the low colonisation rates observed within the *P. lanceolata* roots.

Unexpectedly (in Chapter 4) it was noted that soil within an unplanted environment contained larger aggregates and pores compared to the planted macrocosms. This was because roots utilised pore space (particularly > 10 μm in diameter) and through their physical presence compressed surrounding soil (Braunack and Freebairn, 1988) reducing pore area and fragmenting macro-aggregates into micro-aggregates (that are important in hierarchical aggregation models (Tisdall and Oades, 1982)). In Chapter 5 however, soil from the planted side of the macrocosm had a higher total porosity and mean pore area, due to the biophysical action of roots, resulting in the movement of soil particles binding them together creating additional and larger pore space. This was particularly the case since the roots remained free from becoming root bound as previously observed in Chapter 4, which resulted in the breakdown of aggregates and loss of larger pores. These results from Chapter 5 agree with other measurements in Chapter 4, namely that porosity was significantly higher in the planted non-AMF treatment correlating with columns containing the most root biomass. Soil aggregates under the presence of roots have greater stability (Haynes and Beare, 1997) with root hairs being important in the adhesion and stability of soil compared to fungal hyphae (Moreno-Espíndola *et al*., 2007). In addition, Feeney *et al.* (2006a) also highlighted the

importance of roots (and microbes) in the formation in micropores over a 30 day period. Hence work presented here also leads to the assumption that the driving force behind aggregate formation and stabilisation, in addition to the development of porous spaces within the soil, was predominantly due to root activity.

Aggregate water repellency peaked when root biomass, AMF colonisation and soil biomass were at their highest, suggesting that chemicals such as glomalin, released by AMF and exudates released from microbe functioning and roots, increased the water repellency of soils (Wallis and Horne, 1992; Hallett and Young, 1999; Czarnes *et al*., 2000; De Bano, 2000). Czachor (2006a, b) also discussed the influence of pore characteristics, with non-cylindrical shaped pore influencing wettability and repellency. The increase in water repellency of aggregates can lead to improved and maintained aggregate stability (Piccolo and Mbagwu, 1999), thus improving soil structure over long term periods in addition to increasing the carbon sequestration of soils (Spaccini *et al.,* 2002). However disadvantages include preferential wetting, particularly within the plant zone that influences plant growth through water availability and nutrient losses (Ritsema *et al*., 1993; Dekker and Ritsema, 1996; Doerr, Shakesby and Walsh, 2000; Gordon and Hallett, 2009). In addition to reducing and modifying water infiltration, more extreme effects include increases in surface runoff and soil erosion due to soil water repellency (Doerr, Shakesby and Walsh, 2000).

6.1.4 *Effect of Microbial Communities and Diversity on Soil Structure*

The soil system is extremely important since it controls a vast number of processes such as nutrient cycling and has huge economic value through agriculture, yet despite this there have been very few investigations that have assessed the impact biodiversity has on a soil's structure. Davidson and Grieve. (2006) provided an investigation into the effect of biodiversity on soil structure through modification of fauna *via* additions of different faunal size classes and liming; concluding the loss or change in some species within the soil ecosystem had no measureable effect, since soil has a huge diversity. Their studies (Davidson *et al.,* 2002; Davidson and Grieve, 2006) focused predominantly on macrofauna and their excretions, without thorough assessment of microorganisms, that were highlighted by Tisdall and Oades (1982) as being vital in microaggregate formation and hence ultimately macroaggregate formation.

Chapter 3 highlighted the importance of microbial communities within the soil environment. Total porosity and mean pore size showed a distinct relationship with culturable bacterial and fungal numbers suggesting that as culturable microorganism numbers increased, soil structure measured by total porosity and mean pore area increased. Porosity was found to increase 144.7 % in the clay loam, 18.5 % in the loamy sand and 0.07 % in the sandy loam soil after a 9 month incubation period (after initial soil settling). Feeney *et al*. (2006a) also observed increases in porosity (although over a short time period of 30 days) due to the presence of microbes. Furthermore in Chapter 4 and 5, a relationship between microbial communities and soil structure was observed within the macrocosms inoculated at two different dilution levels. In Chapter

4 bare soil macrocosms amended with the 10^1 dilution had lower species diversity than the 10^6 amended soils (despite both dilutions having the same biomass, which was unexpected; Griffiths *et al.,* 2001). Within this 10^1 dilution-amended bare soil (with lower diversity), porosity, mean pore size and nearest neighbour distance between pores all increased, with sphericity decreasing. Moreover in the planted AMF macrocosms of Chapter 4 the opposite impact of dilution level on bacterial diversity was observed compared to the bare soil macrocosms, with the 10^6 dilution having the least diverse soil in terms of bacteria. The results however concurred with those from the bare soil treatment, demonstrating that in a soil environment with low bacterial diversity mean pore size, pore perimeter and nearest neighbour distance increased and pore sphericity decreased. Thus, it can be assumed that as bacterial diversity within soil systems declines, soil structure development (namely aggregate size and pore morphology) improves.

The impact of microbes on soil structure can also be assessed in terms of bacterial and fungal numbers (similar to that of Chapter 3). Within the bare soil amended with the 10^1 dilution (of Chapter 4), higher bacterial and fungal numbers would be expected (Wertz *et al.,* 2006), hence resulting in the increase in porosity and mean pore size (in addition to other factors) within soil macrocosms. A similar pattern was also observed in Chapter 5, with soil within the 10^1 dilution-amended treatments having a significantly higher total porosity, mean pore size and pore perimeter, assumed to be due to different microbial numbers from inoculation using the dilution technique (Wertz *et al.,* 2006), particularly since no differences in diversity were observed. Furthermore, from T-RFLP profiles (Chapter 4), it was evident that total porosity was

correlated with bacterial species richness, since increased bacterial species richness resulted in greater porosity. Despite porosity and mean pore size being influenced by bacterial species richness, aggregate size and stability were generally more controlled by organic matter content, since aggregate size and stability were greater in the high dilution macrocosms where organic matter was highest. This supports findings by Chaney and Swift (1984) that highlighted an increase in aggregate stability with increasing organic matter and more recently by Chenu, Le Bissonnais and Arrouays (2000) who suggested increased stability with organic matter was related to an increased hydrophobicity.

T-RFLP profiles from Chapter 5 also highlighted the impact microbial diversity has on soil structure, with bacterial and fungal diversity both having a negative impact on aggregate size within the unplanted and planted side of the macrocosms respectively. Furthermore reductions of fungal diversity within the soil macrocosms increased aggregate stability. This relationship assumes that as species diversity declines, microbial processes that release exudates (for example), that aid aggregate formation and stabilisation increase (due to the lack of competition) causing an improvement to aggregate size and stability. A very limited number of studies have examined the role of fungal diversity on soil aggregation with Schreiner *et al. (*1997) illustrating an increase in aggregate stability with diversity and Klironomos *et al. (*2005) finding no significant effect of fungal diversity on aggregate stability. Moreover, generally within the literature it is assumed that redundancy of functions typically exists within the soil environment, especially since no relationship between microbial diversity and decomposition of organic matter has been observed (Griffiths *et al*., 2000 and 2001).

More specific functions such as nitrification and methane oxidation however have been observed to reduce with decreased microbial diversity (Griffiths *et al*., 2000). The results presented here suggest that species diversity losses within the soil may prove to be advantageous for soil structure development, hence suggesting the effect of biodiversity of soil structure may follow the idiosyncratic hypothesis instead. This hypothesis demonstrates that the losses of biodiversity on soil structure would be determined by conditions (e.g. community composition, site fertility and disturbance) under which the extinction occurs, and thus ultimately on the impact the loss of that species has on soil structure.

Since soil structure can be measured in many different ways (e.g. total porosity, mean pore size, aggregate size, stability and water repellency), biodiversity losses may have effects on various aspects of the soil structure. A decline in species diversity may have idiosyncratic effects on processes that predominantly influence total porosity and pore size within a soil, but may be redundant for other soil processes, such as aggregate stability. Since aggregate stability increases with increasing organic matter (Chaney and Swift, 1984); and that there is a high degree of redundancy with regard to decomposition, this may ultimately mean that redundancy may exists in terms of aggregate stability and maintenance, with a reduction of certain species having very little if any affect on aggregate stability. As so many processes are involved in soil structure formation and stabilisation, it seems reasonable that each process which takes place within the soil ecosystem (that influences soil structure) needs to be assessed with regard to the impacts of diversity losses on the function.

In addition to the effect culturable numbers and diversity had on soil structure in Chapter 3, total microbial activity also influenced mean pore area (Chapter 4). This was probably due to the release of exudates from microbes (Czarnes *et al.,* 2000) binding soil particles together forming aggregates and increasing pore area. Since the soil environment is so dynamic, the reverse pattern of this should also be considered, with mean pore area influencing microbial activity within the soil. As illustrated through soil thin sections that had been biologically stained, microbial communities within the soil were related to areas of pore spaces within the soil. Nunan *et al*. (2001) found that bacteria tend to clump near pore spaces, where substrate availability (Wright *et al*., 1995), water, air and nutrient flow would be highest. Thus if pores are larger in size, the area available for microbes to inhabit, which are high in resources, would increase leading to an increase in soil activity.

6.2 EXPERIMENTAL APPROACHES AND METHODOLOGIES

6.2.1 APPLICATION OF DIFFERING BIODIVERSITY LEVELS

From T-RFLP profiles of soil samples described in Chapter 4 and 5, it became apparent that use of differing dilutions did indeed change the microbial communities. These changes were not exactly as expected, with differences in diversity only associated due to the differing soil conditions (i.e. presence of plants roots and variations in AMF species). Such variation in responses by the soil microbial community may be due to insufficient incubation of the soil prior to the start of the experiment, causing incomplete development of an even microbial population (Griffiths *et al.,* 2001 and Wertz *et al*., 2006 left soils for time periods ranging from 19 weeks to 9 months after

inoculation). Therefore the immediate introduction of plants and mycorrhizal fungi to the soil, may have had sufficient effects to modify inoculated soil communities before initial development of different diversity levels. Such limited changes in diversity of denitrifiers and ammonia oxidising bacteria was also observed in low dilution by Wertz *et al. (*2006). However, although limited differences in diversity were observed using the dilution technique described by Griffiths *et al*. (2001), the technique still acted as a suitable methodology for modification of the re-introduced microbial populations within the sterilised soil. Indeed, T-RFLP analysis of inoculated soils left for 14 weeks prior to experimentation in a parallel study at Nottingham University, found no dilution-related decreases in diversity, but did demonstrate alterations in microbial composition of the soils receiving a range of dilutions (West, *Pers. Comm*., 2009).

6.2.2 MICROBIAL ASSESSMENTS

Despite some recent criticism of the use of culturable community assessment, such as plate counts in modern environmental microbial ecology (Ritz, 2007), there still remains a large number of publications reporting CFU data. Indeed within the area of soil research, there is a linear increase in papers being published using CFUs, with more recent work utilising the technique as it as quick and inexpensive compared to DNA methods (after a Web of Science search using 'soil AND CFU' as search terms). Hence, for a simple, inexpensive and quick look at microbial counts within the soil microcosms at varying points in time, CFUs appear to be a suitable option. It is worth noting however that CFUs related to single cells and the culturable conditions do not replicate those of the heterogeneous natural soil environment in which the microorganisms normally grow and function. Ritz (2007) also states that since

organisms have evolved, the culturable community would further be influenced by the presence and status of other organisms within the vicinity of other species.

Biolog microtitre plates, have also come under criticism, since the carbon substrates present within the 95 wells, are not completely relevant to those found in the natural soil system (Konopka, Oliver and Turco, 1998). The well conditions are aqueous, buffered pH-neutral, temperature and light controlled in addition to being nutrient rich. These factors introduce bias into assessments as the metabolic potential of the natural system maybe unrepresented (Smalla *et al*., 1998). Pre-treatment of soil prior to inoculation (through sieving) and the quantity of soil used for inoculation both influence activity within the Biolog plate, particularly with regard to differences in inoculation cell density between plates (Haack *et al.,* 1995; Preston-Mafham, Boddy and Randersoon, 2002). Moreover, Konopka, Oliver and Turco, (1998) also suggest that inoculation from such natural systems may lead to low cell densities being transferred into the well, altering the physiological state of the organisms. The approach is further hampered by the fact it is culture based and slow growing bacteria will have a nominal contribution to the overall profile (Preston-Mafham, Boody and Randerson, 2002). Finally, separate plates are required for bacterial and fungal communities since fungal species are not capable of reducing the purple tetrazolium salt.

Despite some negative reviews of Biolog® plates, it still remains a popular technique for rapid visualisation of microbial communities. For example, 37 publications were found using a Web of Science search of 'Biolog AND soil' during 2009 (correct as of

23th August 2009). The technique has been further developed to allow determination of physiological profiles of whole soil microbial communities through the measurement of carbon dioxide (CO_2) evolved from varying carbon substrates within a microtitre plate (Campbell *et al*., 2003). Hence it remains a rapid method for gaining insight into metabolic potential differences between soil microbial communities, as long as replication (in order to reduce the likelihood of missing rare species within the community), soil inoculum quantity remains constant and correction for background absorbance is applied through transforming with AWCD (Preston-Mafham, Boddy and Randerson, 2002). Such problems associated with culturability of microbes by plate counts and Biolog microtitre plates was resolved through the use of DNA profiling that provided a reliable and rapid methodology through the use of an automated sequencer.

Although reducing the culturability biases, T-RFLP has its own disadvantages (as with any method) particularly since the identification of T-RFLP peaks cannot be made due to the inability to generate sequence information (Anderson and Cairney, 2004). Further drawbacks include the lack of distinction between fragments that share the same fragment length, but different sequences, which is represented by only one peak on the T-RFLP profile. This is particularly the case when T-RFLP is used on complex communities (like soil), where the total diversity within the sample can be compressed to a small number of distinct peaks since phylogentic differences cannot be made (Bibiloni, Lay and Tannock, 2008). Although this problem can be diminished through the use of more than one restriction enzyme. Reduced peak occurrence can lead to oversimplification of the diversity within a sample (as observed with the low number of peaks detected in this study). Furthermore, with such complex ecosystems rare species

may not amplify during PCR preventing detection (Dickie, Xu and Koide, 2002). Since the soil is a relatively uncharacterised environment identification of peaks present within the sampled soil is limited due to the lack of sequence data related to fragments present within the soil under specific restriction enzymes such as HaeII and MseI, in addition to the limited bacterial database currently determined using the 23S region compared to that of 16S.

6.2.3 SOIL STRUCTURAL ANALYSIS

Numerous methods have been used to determine aggregate stability resulting in no universal method for its assessment. In this study a technique described by Le Bissonnais (1996) was used. Le Bissonnais described three methods to determine an aggregate's stability which assessed the four main mechanisms for aggregate breakdown. These were by slaking, breakdown by differential swelling of soil primary particles, mechanical breakdown by raindrop impact and physio-chemical dispersion. Results in this study were determined using the slaking technique which were very reliable and consistent. However, with no universal agreement as to the most appropriate method to study aggregate stability (as discussed in the review by Díaz-Zorita, Perfect and Grove, 2002), comparisons in aggregate stability data from other studies must be assumed with caution.

The use of X-ray CT throughout all experimental work was vital in determining soil structure within the micro- and macrocosms over time non-invasively. Generally this technique provided an excellent tool for soil structure visualisation. A disadvantage of

the technique was the lack of differentiation between soil pore space and root material (due to the close density values that air and root material share) resulting in the overestimation of pore space. The development of micro-X-ray CT has resolved some of these discrimination issues and has even allowed quantifications of root diameter (Gregory *et al*., 2003), something that could in the future be utilised on X-ray CT at the larger scale. Further overall development of this technique could be undertaken through the generation of more algorithms for application in X-ray CT analysis of soil images (such as appropriate techniques for auto-thresholding) to prevent misclassification errors through manual (human error) and allow standardisation across the technique, As mentioned in section 1.1.3.4 this technique is still relatively new, so with further technological advances with regard to image resolution and reconstruction this technique appears to be very useful technique for soil structure determination.

7 CONCLUSIONS AND FUTURE WORK

7.1 CONCLUSIONS

This research has highlighted that interactions in soil are complex with direct and indirect associations taking place at the micro- through to macro- scale. Soil texture influenced soil structural development and microbial communities that lived within the soil ecosystem. Furthermore the presence and type of mycorrhizal fungi within the soil also influenced microbial communities within the soil environment. As highlighted in previous studies the importance of organic matter on aggregate stability was also illustrated, with aggregate stability increasing with increased organic matter content.

It is widely believed that soil structure is affected by microbial communities (despite there being very little data to prove this), however work within this study particularly highlighted the importance of interactions between microbes and plants. Soil structure development was determined through changes in aggregate size, stability and porous characteristics over time determined from X-ray CT. Investigations indicated that after a settling period, soil structural development took place within soil microcosms under the influence of microbial populations alone over a nine month period, whereas within a planted soil (with and without AMF) soil structural development took place over four months. This illustrated that the introduction of plants and their roots to the soil ecosystem accelerated the development of soil structure directly and indirectly (by altering microbial communities), until a point where root density became too high resulting in breakdown of soil structure through their movement and compression of

surrounding soil. However generally it is concluded that root activity is the main driving force behind soil structure development compared to that of microorganisms.

This work provided insights into how soil structure is affected by the diversity of bacteria, saprotrophic fungi and mycorrhizal fungi within planted and unplanted macrocosms. Within planted (but non-mycorrhizal) treatments, less biodiverse soil (in terms of microorganisms) increased aggregate size, whereas within unplanted and planted soil with mycorrhizal fungi porosity and pore size were larger within the less diverse soil. However, the reverse effects of diversity on aggregate stability were observed, where declines in biodiversity, caused reductions in organic matter content and thus declines in aggregate stability. Soil has generally been characterised by a redundancy of function and generally a reduction in microbial diversity has little effect on overall processes. However with regard to the physical structure, the effect of biodiversity loss on function may in fact be idiosyncratic, as the effects of biodiversity losses appear to be influenced by soil conditions (such as the presence or not of plants and mycorrhizal fungi). Furthermore it was illustrated that with a slight decline in diversity, an improvement in soil structure was observed; possibly because other microorganisms effectively take on the function of the extinct microbe. In order to assess the true extent biodiversity has on soil structure as a whole, thorough assessment of the functions and the species involved in the development and stabilisation of soil is fully required. This should be undertaken before the assessment of the effect biodiversity losses have on each of these individual functions. Furthermore it was also observed that as microbial numbers increased soil structure improved, highlighting that

a soil system high in microbial numbers, but with a low diversity maybe be appropriate for efficient development and stabilisation of soil structure.

7.2 IMPLICATIONS

With changes and extinctions within the soil microbial community, due to agricultural practices and intensification in addition to changes mediated by climate change, the impact of microbial numbers (and biodiversity) on soil structure and thus plant productivity is highly important. This work has highlighted the importance of bacterial and fungal numbers on soil structural measurements, namely porosity and mean pore size, thus any decline in the numbers of microbes within a soil community could have an impact on soil structure and ultimately agricultural productivity.

To humans, sustaining soil structure is vital to maintaining crop production in addition to grazing pastures, particularly since the soil environment provides all the food components humans require including carbohydrates, proteins, fibre, water, minerals and vitamins. Estimates from the US highlighted that up to 99 % of US food is sourced from the soil environment (Pimental and Giampietro, 1994); hence the maintenance of this ecosystem is vital. In recent years food security has become a very important concern, particularly within developing countries, but even within developed countries like the UK which are open to trade. Food security is defined as when all people, at all times, have access to sufficient, safe and nutritious food to meet their dietary need and food preferences for an active and healthy life (FAO, 1996). As global population increases, it is estimated that by 2050 the world population will be 8.9 billion (Chamie,

2004) thus food security for the future is an important issue. With cereal deficits in developing countries predicted to increase from 78 to 244 million metric tons by 2020 (Oldeman, 1998), it is vital that the soil environment and its structure is maintained in order to maintain the agricultural demand placed on it. Improving management of soil organic matter (that improves soil aggregation and stability) can double the financial returns of UK farmers from £31 to £66 per hectare, due to easier tillage, fertiliser saving and higher yields (Defra, 2008), thus proper management of such ecosystems is fundamental for both food security and effective soil function to maintain this system.

Climate change will also have important impacts on food and farming, particularly on what kinds of farming and what crops can be grown in certain areas. The preservation of a good soil structure will be fundamental to maintaining good drainage within soils, good seedbeds (Bouaziz and Bruckler, 1989; Souty and Rode, 1993; Aubertot *et al.,* 1999) and a suitable environment for crop anchorage (Mooney *et al*., 2007), especially with the occurrence of extreme weather events (e.g. wind, rain and floods) likely to increase (Barling, Sharpe and Lang, 2008). Changes in temperature and rainfall patterns, will also influence microbial populations, resulting in some adaption but also extinction, that will influence ecosystem functioning. Furthermore awareness and modification of current and modern farming practices is vital in the management of soil throughout these changing environmental conditions (Defra, 2008).

In addition to sustaining food production, the porous network within soil controls the flow of water and chemical substances between the atmosphere and the earth, in addition to acting as a source and store for gases in the atmosphere. Soil contains up to

58 % carbon (Defra, 2008), with 3.73 million tonnes (Mt) of carbon emitted from UK soils and 1.52 Mt added in 2003 (Barling, Sharpe and Lang, 2008). Thus maintenance of such an ecosystem is vital for the continued functioning of this ecosystem within water and gaseous flow cycles. Effective management of the soil, particularly within the agricultural context (West and Post, 2002) may also improve the potential of the soil environment to sequester carbon dioxide from the atmosphere (Sampson and Scholes, 2000) partially mitigating atmospheric carbon dioxide, albeit at a minor scale.

7.3 FUTURE WORK

Investigations on biodiversity/soil-structure interactions must focus on effective methodologies for manipulating diversity that provide realistic changes pertinent to the natural environment. Furthermore, effective studies need to be conducted in order to apply results from laboratory based studies to the field environment where large areas need to be assessed.

In addition to the research presented here, which could be improved through use of more replication in chapter 3 and selection of more restriction enzymes for T-RFLP in chapters 4 and 5, other investigations are required in order to assess the true impact biodiversity has within the highly important soil environment.

- A complimentary study to those presented within this thesis would also include an assessment *of a soil structural response to* ***declining*** *levels of microbial diversity*. Throughout Chapters 3, 4 and 5 the effect of diversity on soil

structural development was assessed, however a comparison would be to monitor the effect of biodiversity decline on intact soil cores taken invasively from the field and measured using non-destructive techniques such as X-ray CT.

- *Determination of the impact microorganisms have on soil structure at the micro-scale.* Research within this study was limited to a resolution of ~ 65 µm pixel^{-1}, hence further assessment at a much enhanced resolution may be able to monitor finer changes to soil structure over time than those observed here. It is hypothesised that the pore space and pore connectivity within an aggregate may influence the stability of that aggregate (Papadopoulos, 2007). Hence an unstable aggregate would have pores poorly connected with each other and the outside environment, whereas pore space within stable aggregates would be well connected both within and externally. A study could be undertaken where microbial activity within individual aggregates would be manipulated and the effect of that aggregate's pore structure (through X-ray µCT) and stability monitored over time (particularly since aggregates have been recognised to be held together under transient, temporary and persistent binding agents that vary in the timescale in which they influence aggregate stability). These factors could ultimately influence microbial biodiversity, particularly since microbes tend to be located near pore space (Nunan *et al*., 2001). A further complimentary study could look at the converse of this idea with an assessment of microbial communities (by T-RFLP while using more than one restriction

enzymes) within aggregates, comparing systems in relation to their porous environment (i.e. connectivity within and externally).

- *Comprehensive assessments of the effect bacterial and fungal numbers have on soil structure.* It has been observed and hypothesised during the current research and by Aşkin *et al. (*no date), that as microbial numbers increase soil structural development increases. However, no thorough investigation today has been focused solely on this hypothesis. A macrocosm investigation into the effect different bacterial and fungal numbers have on the soil could be undertaken, by inoculating sterilised soil with various microbe numbers before assessment of the aggregate's size and stability and porous network is made with the use of X-ray μCT. Furthermore during this study a comprehensive assessment of the functioning of the particular bacteria and fungi present within the inoculated soils could be determined, to actually assess the impact various organisms have on soil function (which currently is one of the main problems affecting our knowledge of the impact biodiversity has on soil functioning).

- *An assessment of the capability of soil to support a decline in microbial diversity*. The current investigation clearly demonstrated that biodiversity can have an impact on soil, particularly within the bare soil environment, however a more through quantification of the effects biodiversity has on long term soil structure is required. Due to time constraints the longest time period soil structure was assessed over was 15 months, since soil structure can take years to develop, a longer assessment would be required.

BIBLIOGRAPHY

ABBOTT, L.K. AND ROBSON, A.D. (1985) Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytologist,* **99**, 245–255.

ABBOTT, L.K., ROBSON, A.D. AND DE BOER, G. (1984) The effect of phosphorus on the formation of hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus Fasciculatum*. *New Phytologist*, **97**, 437-446.

ABO-GHALIA, H.H. AND KHALAFALLAH, A.A. (2008) Responses of wheat plants associated with arbuscular mycorrhizal fungi to short-term water stress followed by recovery at three growth stages. *Journal of Applied Sciences Research*, **4**, 570-580.

ACINAS, S., SARMA-RUPAVTARM, R., KLEPAC-CERAJ, V. AND POLZ, M.F. (2005) PCR-induced sequence artefacts and bias: Insights from Comparison of two 16S rRNA clone libraries constructed from the same sample. *Applied Environmental Microbiology*, **71**, 8966-8969.

AKIN, S. AND KOVSCEK, A.R. (2003) Computed tomography in petroleum engineering research. *In*: MEES, F., SWENNEN, R., VAN GEET, M. AND JACOBS, P. (EDS.) Applications of X-ray computed tomography in the geosciences, p 23-38. Geological Society, Special Publication 215, Pp243.

AL-KARAKI, G., MCMICHAEL, B. AND ZAK, J. (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza*, **14**, 263-269.

AMANN, R.I., LUDWIG, W. AND SCHLEIFER, K.H. (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**, 143-169.

AMELLAL, N., BURTIN, G., BARTOLI, F. AND HEULIN, T. (1998) Colonization of wheat roots by an exopolysaccharide-producing *Pantoea agglomerans* strain and its effect on rhizosphere soil aggregation. *Applied and Environmental Microbiology*, **64**, 3740-3747.

AMES, R.N., INGHAM, E.R. AND REID, C.P.P. (1982) Ultraviolet-induced autofluorescence of arbuscular mycorrhizal root infections: an alternative to cleaning and staining methods for assessing infections. *Canadian Journal of Microbiology*, **28**, 351-355.

AMÉZKETA, E. (1999) Soil aggregate stability: A review. *Journal of Sustainable Agriculture*, **14 (2/3)**, 83-151.

AMÉZKETA, E. AND ARAGÜÉS, R. (1995) Flocculation-dispersion behaviour of arid-zone soil clays as affected by electrolyte concentration and composition. *Investigacion Agraria. Produccion y Proteccion Vegetales (Espana),* **10,** 101-112. Referenced by: AMÉZKETA, E. (1999) Soil aggregate stability: A review. *Journal of Sustainable Agriculture*, **14** (2/3), 83-151.

AMÉZKETA, E., SINGER, M.J., AND LE BISSONNAIS, Y. (1996) Testing a procedure for measuring water-stable-aggregation. *Soil Science Society America Journal*, **60**, 888-894.

ANDERSON, I.C. AND CAIRNEY, J.W.G. (2004) Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. *Environmental Microbiology*, **6**, 769-779.

ANDERSON, J.P.E. AND DOMSCH, K.H. (1978) A physiological method for quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, **10**, 215-221.

ANDERSON, S.H., PEYTON, R.L. AND GANTZER, C.J. (1990) Evaluation of constructed and natural soil macropores using X-ray computed tomography. *Geoderma*, **46**, 13-29.

ANDRADE, G., MIHARA, K.L., LINDERMAN, R.G. AND BETHLENFALVAY, G.J. (1997) Bacteria from rhizosphere and hydrosphere soils of different arbuscular-mycorrhizal fungi. *Plant and Soil*, **192**, 71-79.

ANDRADE, G., MIHARA, K.L., LINDERMAN, R.G. AND BETHLENFALVAY, G.J. (1998) Bacterial associations with the mycorrhizosphere and hyphosphere of the arbuscular mycorrhizal fungus *Glomus mosseae*. *Plant and Soil,* **202**. 79–81.

ANDRÉ, H.M., DUCARME, X. AND LEBRUN, P. (2002) Soil biodiversity: myth, reality or conning? *OIKOS*, **96**, 3-24.

ANDREEV, V.Y., GENEROZOVA, I.P. AND VARTAPETIAN, B.B. (1991) Energy status and mitochondrial ultrastructure of excised pea root at anoxia and postanoxia. *Plant Physiology and Biochemistry*, **29**, 171-176.

ANGERS, D.A. AND CARON, J. (1998) Plant-induced changes in soil structure: Processes and feedbacks. *Biogeochemistry*, **42**, 55-72.

ANTHONY, R.M., BROWN, T.J. AND FRENCH, G.L. (2000) Rapid diagnosis of bacteria by universal amplification of 23S ribosomal DNA followed by hybridization to an oligonucleotide array. *Journal of Clinical Microbiology*, **38**, 781-788.

ARTURSSON, V., FINLAY, R.D. AND JANSSON, J.K. (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environmental Microbiology*, **8 (1)**, 1-10.

ARTURSSON, V. AND JANSSON, J.K. (2003) Use of bromodeoxyuridine immunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae. *Applied and Environmental Microbiology,* **69,** 6208–6215.

AŞKIN, T., GÜLSER, C., KIZILKAYA, R. AND ÖZDEMIRE, N. (no date) The effects of inoculation of soil with different numbers of bacteria on aggregations. Soil Science Society of Turkey. Found at: www.toprak.org.tr/isd/isd_43.htm (Viewed: 1/6/09).

ATKINSON, B.S. (2008) Identification of optimum seedbed preparation and establishment using soil structural visualisation. PhD thesis, University of Nottingham.

ATKINSON, B.S., SPARKES, D.L. AND MOONEY, S.J. (2009) Effect of seedbed cultivation and soil macrostructure on the establishment of winter wheat (*Triticum aestivum*). *Soil and Tillage Research*, **103**, 291-301.

ATLAS, R.M. AND BARTHA, R. (1987) Microbial ecology: Fundamentals and applications, The Benjamim/Cummings Publishing Company, Inc., California. Pp 640.

AUBERTOT, J.N., DÜRR, C., KIÊU, K. AND RICHARD, G. (1999) Characterization of sugar beet seedbed structure. *Soil Science Society of America Journal*, **63**, 1377-1384.

AVIO, L., PELLEGRIONO, E., BONARI, E. AND GIOVANNETTI, M. (2006) Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradial mycelia networks. *New Phytologist*, **172**, 347-357.

AZCON, R. (1987) Germination and hyphal growth of *Glomus mosseae* in vitro: effect of rhizosphere bacteria and cell free culture media. *Soil Biology and Biochemistry*, **19**, 417-419.

AZĆON, R. AND OCAMPO, J.A. (1981) Factors affecting the vesicular arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. *New Phytologist*, **87**, 677-685.

BÅÅTH, E., DÍAZ-RAVIÑA, M., FROSTEGÅRD, Å. AND CAMPBELL, C.D. (1998) Effect of metal-rich sludge amendments on the soil microbial community. *Applied Environmental Microbiology*, **64**, 238-245.

BACOT, C.M. AND REEVE, R.H. (1991) Novel RNA gene organization in the 16S-23S intergenic spacer of the *Streptococcus pneumoniae* rRNA gene clusters. *Journal of Bacteriology*, **173**, 4234-4236.

BAGO, B., CANO, C., AZCȯN-AGUILAR, C., SAMSON, J., COUGHLAN, A.P. AND PICHÉ, Y. (2004) Differential morphogenesis of the extraradical mycelium of an arbuscular mycorrhizal fungus grown monoxenically on spatially heterogeneous culture media. *Mycologia*, **96**, 452-463.

BANSAL, M. AND MUKERJI, K.G. (1994) Positive correlation between VAM-induced changes in root exudation and mycorrhizosphere mycoflora. *Mycorrhiza*, **5**, 39-44.

BARDGETT, R. (2005) The biology of soil: A community and ecosystem approach. Oxford University Press, UK. Pp 242.

BARLING, D., SHARPE, R. AND LANG, T. (2008) Rethinking Britain's food security. Soil Association. Available at: http://www.soilassociation.org/LinkClick.aspx?fileticket=wCYoHYSHsy8%3d&tabid=387 (Viewed: 29/8/09). Pp 46.

BARROS, N., FEIJOȯ, S. AND BALSA, R. (1997) Comparative study of the microbial activity in different soils by the microcalorimetric method. *Thermochimica Acta*, **296**, 53-58.

BARRY, T., COLLERAN, G., GLENNON, M., DUNICAN, L.K. AND GANNON, F. (1991) The 16/23S ribosomal spacer region as target for DNA probes to identify eubacteria. *PCR Methods and Applications*, **1**, 51-56.

BASTARDIE, F., CAPOWIEZ, Y. AND CLUZEAU, D. (2005) 3D characterisation of earthwork burrow systems in natural soil cores collected from a 12-year-old pasture. *Applied Soil Ecology*, **30**, 34-46.

BAUDOIN, E., BENIZRI, E. AND GUCKERT, A. (2002) Impact of growth stage on the bacteria community structure along maize roots, as determined by metabolic and genetic fingerprinting. *Applied Soil Ecology*, **19**, 135-145.

BAYLIS, G.T.S. (1975) The magnoloid mycorrhiza and mycotrophy in root systems derived from it. *In*: SANDERS, F.F. AND TINKER, P.B. (EDS.) *Endomycorrhizae.* Academic Press, London, p.373-389.

BEARDEN, B.N. (2001) Influence of arbuscular mycorrhizal fungi on soil structure and soil water characteristics of vertisols. *Plant and Soil*, **229**, 245-258.

BEARDEN, B.N. AND PETERSEN, L. (2000) Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of a vertisol. *Plant and Soil*, **218**, 173-183.

BENGOUGH, A.G., CROSER, C. AND PRITCHARD, J. (1997) A biophysical analysis of root growth under mechanical stress. *Plant and Soil*, **189**, 155-164.

BENGOUGH, A.G. AND MACKENZIE, C.J. (1994) Simultaneous measurement of root force and elongation for seedling pea roots. *Journal of Experimental Botany*, **45**, 95-102.

BIANCIOTTO, V., BANDI, C., MINERDI, D., SIRONI, M., TICHY, H.V. AND BONFANTE, P. (1996) An obligately endosymbiotic mycorrhizal fungus itself harbours obligately intracellular bacteria. *Applied Environmental Microbiology*, **62**, 3005-3010.

BIBILONI, R., LAY, C. AND TANNOCK, G.W. (2008) Probiotics: Lessons learned from nucleic acid-based analysis of bowel communities. *In*: COCOLIN, L. AND ERCOLINI, D. Molecular Techniques in the microbial ecology of fermented foods. Springer, New York, USA, p 225-244.

BLANKE, V., RENKER, C., WAGNER, M., FULLNER, K., HELD, M., KUHN, A.J. AND BUSCOT, F. (2005) Nitrogen supply affects arbuscular mycorrhizal colonisation of *Artemisia vulgaris* in a phosphate-polluted field site. *New Phytologist*, **166**, 981-992.

BOERSMA, O.H. AND KOOISTRA, M.J. (1994) Differences in soil structure of silt loam Typic Fluvaqents under various agricultural management practices. *Agriculture, Ecosystems and Environment*, **51**, 21-42.

BOIX-FAYOS, C., CALVO-CASES, A., IMESON, A.C. AND SORIANO-SOTO, M.D. (2001) Influence of soil properties on the aggregation of some Mediterranean soils and the use of aggregate size and stability as land degradation indicators. *Catena*, **44**, 47-67.

BOLAN, N.S. (1991) A critical review of mycorrhizae fungi in the uptake of phosphorus by plants. *Plant and Soil*, **134**, 189-207.

BOSSIO, D.A. AND SCOW, K.M. (1995) Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. *Applied and Environmental Microbiology*, **61**, 4043-4050.

BOSSUYT, H., DENEF, K., SIX, J., FREY, S.D., MERCKX, R. AND PAUSTIAN, K. (2001) Influence of microbial populations and residue quality on aggregate stability. *Applied Soil Ecology*, **16**, 195-208.

BOUAZIZ, A. AND BRUCKLER, L. (1989) Modelling wheat seedling growth and emergence: II. Comparison with field experiments. *Soil Science Society of America Journal*, **53**, 1838-1846.

BOUMA, J., JONGERIUS, A., BOERSMA, O., JAGER, A. AND SCHOONDERBEEK, D. (1977) The function of different types of macropores during saturated flow through four swelling soil horizons. *Soil Science Society of America Journal*, **41**, 945-950.

BOWEN, H.D. (1981) Alleviating mechanical impedance. *In*: ARKIN, G.F. AND TAYLOR, H.M. (EDS.) Modifying the root environment to reduce crop stress. American Society of Agricultural Engineers, Michigan, USA. Pp 412.

BRAUNACK, M.V.A. AND FREEBAIRN, D.M. (1988) The effect of bulk density on root growth. In: *Proceedings of the 11th International Conference of the International Soil Tillage Research Organisation, Volume I*, p 25-30. Edinburgh, Scotland: ISTRO. Referenced by YOUNG, I.M. (1998) Biophysical interactions at the root-soil interface: a review. *Journal of Agricultural Science*, **130**, 1-7.

BRONICK, C.J. AND LAL, R. (2005) Soil structure and management: a review. *Geoderma*, **124**, 3-22.

BROOKES, P.C., KRAGT, J.F., POWLSON, D.S. AND JENKINSON, D.S. (1985) Chloroform fumigation and the release of soil nitrogen: The effects of fumigation time and temperature. *Soil Biology and Biochemistry*, **17**, 831-835.

BROOKES, P.C., POWLSON, D.S. AND JENKINSON, D.S. (1982) Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry*, **14**, 319-329.

BROWN, D.P., PRATWUM, T.K., BLEDSOE, C., FORD, E.D. AND COTHERN, J.S. (1991) Noninvasive studies of conifer roots: nuclear magnetic resonance (NMR) imaging of Douglas fir seedlings. *Canadian Journal of Forestry Research,* **21**, 1559–1566.

BRUND, A., COUSIN, I., NICOULLAUD, B., DUVAL, O. AND BEGON, J.C. (1996) Backscattered electron scanning images of soil porosity for analyzing soil compaction around roots. *Soil Science Society of America Journal*, **60**, 895-901.

BRUNDRETT, M.C., PICHE, Y. AND PETERSON, R.L. (1984) A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Canadian Journal of Botany*, **62**, 2128-2134.

BRUNEAU, P.M.C., DAVIDSON, D.A., GRIEVE, I.C., YOUNG, I.M. AND NUNAN, N. (2005) The effect of soil horizons and faunal excrement on bacterial distribution in an upland grassland soil. *FEMS Microbiology Ecology*, **52**, 139-144.

BUI, E.N. (1991) Applications of image analysis to soil micromorphology. *Agriculture, Ecosystems and Environment*, **34**, 305-313.

BULL, A.T. (1992) Microbial diversity and the problems of microbial identification. *Fresenius' Journal of Analytical Chemistry*, **343**, 46.

BULLOCK, P., FEDOROFF, N., JONGERIUS, A., STOOPS, G. AND TURSINA, T. (1985) Handbook for soil thin section description. Waine Research Publications. Pp 152.

BUNDY, J.G., PATON, G.I. AND CAMPBELL, C.D. (2002) Microbial communities in different soil types do not converge after diesel contamination. *Journal of Applied Microbiology*, **92**, 276-288.

CAMBARDELLA, C.A. (2006) Aggregation and organic matter. *In:* LAL, R. (ED.) Encyclopaedia of soil science: Volume 1. Second edition. Taylor & Francis (CRC Press). Pp 1959.

CAMPBELL, C.D., CHAPMAN, S.J., CAMERON, C.M., DAVIDSON, M.S. AND POTTS, J.M. (2003) A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Applied and Environmental Microbiology*, **69**, 3593-3599.

CAPOWIEZ, Y., PIERRET, A. AND MORAN, J. (2003) Characterisation of the three-dimensional structure of earthworm burrow systems using image analysis and mathematical morphology. *Biology and Fertility of Soils*, **38**, 301-310.

CARON, J., KAY, B.D. AND PERFECT, E. (1992) Short-term decrease in soil structural stability following bromegrass establishment on a clay loam soil. *Plant and Soil*, **145**, 121-130.

CARTER, M.R. (2004) Researching structural complexity in agricultural soils. *Soil and Tillage Research*, **79**, 1-6.

CARTER, M.R., GREGORICH, E.G., ANGERS, D.A., BEARE, M.H., SPARLING, G.P., WARDLE, D.A. AND VORONEY, R.P. (1999) Interpretation of microbial biomass measurements for soil quality assessment in humid temperate regions. *Canadian Journal of Soil Science*, **79**, 507-520.

CAVAGNARO, T.R., GAO, L.-L., SMITH, F.A. AND SMITH, S.E. (2001) Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytologist*, **151**, 469-475.

CHAMIE, J. (2004) World population prospects: The 2002 revision. Volume III: Analytical report. United Nations, New York. Available at: http://iis-db.stanford.edu/evnts/3961/Joe_Chamie_Population_Prospects_2002.pdf (Viewed: 29/8/09).

CHANEY, K. AND SWIFT, R.S. (1984) The influence of organic matter on aggregate stability in some British soils. *Journal of Soil Science*, **35**, 223-230.

CHANDER, K., KLEIN, T., EBERHARDT, U. AND JOERGENSEN, R.G. (2002) Decomposition of carbon 14-labelled wheat straw in repeatedly fumigated and non-fumigated soils with different levels of heavy metal contamination. *Biology and Fertile Soils*, **35**, 86-91.

CHAPLIN III, F.S., ZAVALETA, E.S., EVINER, V.T., NAYLOR, R.L., VITOUSEK, P.M., REYNOLDS, H.L., HOOPER, D.U., LAVOREL, S., SALA, O.E., HOBBIE, S.E., MACK, M.C. AND DÍAZ, S. (2000) Consequences of changing biodiversity. *Nature*, **405**, 234-242.

CHENU, C. (1989) Influence of a fungal polysaccharide, scleroglucan, on clay microstructures. *Soil Biology and Chemistry*, **21** (2), 299-305.

CHENU, C. (1993) Clay- or sand polysaccharide associations as models for the interface between micro-organisms and soil-water related properties and micro-structures. *Geoderma*, **56**, 143-156.

CHENU, C., LE BISSONNAIS, Y. AND ARROUAYS, D. (2000) Organic matter influence on clay wettability and soil aggregate stability. *Soil Science Society of America Journal*, **64**, 1479-1486.

CHIARINI, L., BEVIVINO, A., DALMASTRI, C., NACAMULLI, C. AND TABACCHIONI, S. (1998) Influence of plant development, cultivar and soil type on microbial colonisation of maize roots. *Applied Soil Ecology*, **8**, 11-18.

CHRISTENSEN, B.T. (1992) Physical fractionation of soil and organic matter in primary particle size and density separates. *Advancements in Soil Science*, **20**, 2-76.

CLAPP, J.P., HELGASON, T., DANIELL, T.J. AND YOUNG, J.P.W. (2002) Genetic studies of the structure and diversity of arbuscular mycorrhizal fungal communities, p. 201-224. *In:* VAN DER HEIJDEN, M.G.A. AND SANDERS, I.R. (EDS.) Mycorrhizal ecology. Springer-Verlag, Berlin, Germany.

CLARK, L.J., WHALLEY, W.R. AND BARRACLOUGH, P.B. (2003) How do roots penetrate strong soil? *Plant and Soil*, **255**, 93-104.

CLEMENT, B.G., KEHL, L.E., DEBORD, K.L. AND KITTS, C.L. (1998) Terminal restriction fragment patterns (TRFLPs), a rapid, PCR based method for the comparison of complex bacterial communities. *Journal of Microbial Methods*, **31**, 135-142.

CNUDDE, V., MASSCHAELE, B., DIERICK, M., VLASSENBROECK, J., VAN HOOREBEKE, L. AND JACOBS, P. (2006) Recent progress in X-ray CT as a geosciences tool. *Applied Geochemistry*, **21**, 826-832.

COLLIER, S.C., YARNES, C.T. AND HERMAN, R.P. (2003) Mycorrhizal dependency of Chihuahuan Desert plants is influenced by life history strategy and root morphology. *Journal of Arid Environments*, **55**, 223-229.

COPETTA, A., LINGUA, G. AND BERTA, G. (2006) Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum L.* var. *Genovese. Mycorrhiza*, **16**, 485-494.

COSENTINO, D., CHENU, C. AND LE BISSONNAIS, Y. (2006) Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil. *Soil Biology and Biochemistry*, **38**, 2053-2062.

CRUZ, C., GREEN, J.J., WATSON, C.A., WILSON, F. AND MARTINS-LOUÇÃO, M.A. (2004) Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient capacity. *Mycorrhiza*, **14**, 177-184.

CULMAN, S.W., GAUCH, H.G., BLACKWOOD, C.B. AND THIES, J.E. (2008) Analysis of T-RFLP data using analysis of variance and ordination methods: A comparative study. *Journal of Microbiological Methods*, **75**, 55-63.

CZACHOR, H. (2006a) Modelling the effect of pore structure and wetting angles on capillary rise in soils having different wettabilities. *Journal of Hydrology*, **328**, 604-613.

CZACHOR, H. (2006b) Modelling the impact of pore topology on the subcritical soil repellency. *Abstract of papers*, Biohydrology Conference 2006, Prague, Czech Republic. 22-24th Sept 2006.

CZARNES, S., HALLETT, P.D., BENGOUGH, A.G. AND YOUNG, I.M. (2000) Root- and microbial-derived mucilages affect soil structure and water transport. *European Journal of Soil Science*, **51**, 435-443.

DAFT, M.J. AND HACSKAYLO, E. (1977) Growth of endomycorrhizal red maple seedlings in sand and anthracite spoil. *Forensic Science,* **23**, 207–216.

DAHLLÖF, I. (2002) Molecular community analysis of microbial diversity. *Current Opinion in Biotechnology*, **13**, 213-217.

DALMASTRI, C., CHIATINI, L., CANTALE, C., BEVIVINO, A. AND TABACCHIONI, S. (1999) Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. *Microbial Ecology*, **38**, 273-284.

DAVIDSON, D.A., BRUNEAU, P.M.C., GRIEVE, I.C., AND YOUNG, I.M. (2002) Impacts of fauna on an upland grassland soil as determined by micromorphological analysis. *Applied Soil Ecology*, **20 (2)**, 133-143.

DAVIDSON, D.A. AND GRIEVE, I.C. (2006) Relationship between biodiversity and soil structure and function: Evidence from laboratory and field experiments. Applied Soil Ecology, **33 (2)**, 176-185.

DAVIDSON, J. (1999) Genetic exchange between bacteria in the environment. *Plasmid*, **42**, 73-91.

DE BANO, L.F. (2000) Water repellency in soils: A historical overview. *Journal of Hydrology*, **231- 232**, 4-32.

DEFRA (2008) Consultation on the draft soil strategy for England. March, 2008. Department for Environment, Food and Rural Affairs, London. Available at: http://webarchive.nationalarchives.gov.uk/20081023133800/http://www.defra.gov.uk/corporate/consult/soilstrategy/consultation.pdf (Viewed: 29/8/09).

DEGENS, B. (1997) Macro-aggregation of soil by biological bonding and binding mechanisms and the factors affecting these: a review. *Australian Journal of Soil Research*, **35**, 431-459.

DEGENS, B.P. (1998) Decreases in microbial functional diversity do not result in corresponding changes in decomposition under different moisture conditions. *Soil Biology and Biochemistry*, **30**, 1989-2000.

DEGENS, B., SPARLING, G.P. AND ABBOTT, L.K. (1994) The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long-term clover-based and grass pastures. *European Journal of Soil Science*, **45**, 459-468.

DE GRYZE, S., SIX, J., BRITS, C. AND MERCKX, R. (2005) A quantification of short term macroaggregate dynamics: influence of wheat residue input and texture. *Soil Biology and Biochemistry*, **37**, 55-66.

DE GRYZE, S., SIX, J. AND MERCKX, R. (2006) Quantifying water-stable soil aggregate turnover and its implication for soil organic matter dynamics in a model study. *European Journal of Soil Science*, **57**, 693-707.

DEKKER, L.W. AND RITSEMA, C.J. (1996) Preferential flow paths in a water repellent clay soil with grass cover. *Water Resources Research*, **32**, 1239-1249.

DENEF, K., SIX, J., BOSSUYT, H., FREY, S.D., ELLIOTT, E., MERCKX, R. AND PAUSTIAN, K. (2001) Influence of dry-wet cycles on the interrelationship between aggregate, particulate

organic matter, and microbial community dynamics. *Soil Biology and Biochemistry*, **33**, 1599-1611.

DENEF, K., SIX, J., MERCKX, R. AND PAUSTIAN, K. (2002) Short-term effects of biological and physical forces on aggregate formation in soils with different clay mineralogy. *Plant and Soil*, **246**, 185-200.

DEXTER, A.R. (1987) Compression of soil around roots. *Plant and Soil*, **97**, 401-406.

DEXTER, A.R. (1988) Advances in characterization of soil structure. *Soil and Tillage Research*, **11**, 199-238.

DÍAZ-ZORITA, M., PERFECT, E. AND GROVE, J.H. (2002) Disruptive methods for assessing soil structure. *Soil and Tillage Research*, **64**, 3-22.

DICKIE, I.A., XU, B. AND KOIDE, R.T. (2002) Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist*, **156**, 527-535.

DICKINSON, C.H., AUSTIN, B. AND GOODFELLOW, M. (1975) Quantitative and qualitative studies of phylloplane bacteria from *Lolium perenne*. *Journal of General Microbiology*, **91**, 157-166.

DODD, J.C., BODDINGTON, C.L., RODRIGUEZ, A., GONZALEZ-CHAVEZ, C. AND MANSUR, I. (2000) Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: Form, function and detection. *Plant and Soil*, **26**, 131-151.

DOERR, S.H., SHAKESBY, R.A. AND WALSH, R.P.D. (2000) Soil water repellency: Its causes, characteristics and hydro-geomorphological significance. *Earth Science Reviews*, **51**, 33-65.

DORAN, J.W. AND PARKIN, T.B. (1994) Defining and assessing soil quality. *In*: DORAN, J.W., COLEMAN, D.C., BEZDICEK, D.F. AND STEWARD, B.A. (EDS.) Defining soil quality for a sustainable environment, Special publication No. 35, Soil Science Society of America, Inc. Madison, WI, USA, p 3-21.

DORAN, J.W. AND ZEISS, M.R. (2000) Soil health and sustainability: Managing the biotic component of soil quality. *Applied Soil Ecology*, **15**, 3-11.

DREES, L.R., WILDING, L.P., KARATHANASIS, A.D. AND BLEVINS, R.L. (1994) Micromorphological characteristics of long-term no-till and conventionally tilled soils. *Soil Science Society of America*, **58**, 508-517.

DREW, M.C. (1997) Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 223-250.

DREW, M.C. AND LYNCH, J.M. (1980) Soil anaerobiosis, microorganisms and root function. *Annual Review of Phytopathology*, **18**, 37-66.

DRURY, C.F., STONE, J.A. AND FINDLAY, W.I. (1991) Microbial biomass and soil structure associated with corn, grasses and legumes. *Soil Science Society of America Journal*, **55**, 805-811.

EDEL-HERMANN, V., DREUMONT, C., PÉREZ-PIQUERES, A. AND STEINBERG, C. (2004) Terminal restriction fragment length polymorphism analysis of ribosomal RNA genes to assess changes in fungal community structure in soils. *FEMS Microbiology Ecology*, **47**, 397-404.

EDGERTON, D.L., HARRIS, J.A., BIRCH, P. AND BULLOCK, P. (1995) Linear relationship between aggregate stability and microbial biomass in three restored soils. *Soil Biology and Biochemistry*, **27**, 1499-1501.

EISSENSTAT, D.M. (1992) Costs and benefits of constructing roots of small diameter. *Journal of Plant Nutrition*, **15**, 763-782.

EISSENSTAT, D.M., GRAHAM, J.H., SYVERTSEN, J.P. AND DROUILLARD, D.L. (1993) Carbon economy of sour orange in relation to mycorrhizal colonisation and phosphorus status. *Annals of Botany*, **71**, 1-10.

ELLIOTT, E.T. (1986) Hierarchic aggregate structure and organic C, N, and P in native and cultivated grassland soils. *Soil Science Society of America Journal*, **50**, 627-633.

ELLIOTT, E.T. AND COLEMAN, D.C. (1988) Let the soil work for us. *Ecological Bulletin*, **39**, 23-32.

ENDLWEBER, K. AND SCHEU, S. (2006) Establishing arbuscular mycorrhiza-free soil: A comparison of size methods and their effects on nutrient mobilization. *Applied Soil Ecology*, **34**, 276-279.

ENVIRONMENT AGENCY (2008) Think soils: Soil assessment to avoid erosion and runoff. Environment Agency, Bristol, UK. Pp 122.

EOM, A.-H., HARTNETT, D.C. AND WILSON, G.W.T. (2000) Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia*, **122**, 435-444.

FANG, M., KREMER, R.J., MOTAVALLI, P.P. AND DAVIS, G. (2005) Bacterial diversity in rhizosphere of nontransgenic and transgenic corn. *Applied and Environmental Microbiology*, **71**, 4132-4136.

FAO (1996) Rome declaration on world food security. Food and Agriculture Organisation, Rome. Available at: http://www.fao.org/docrep/003/w3613e/w3613e00.HTM (Viewed: 29/8/09).

FEENEY, D.F. (2004) Impact of fungi upon soil water relations. PhD thesis. University of Abertay, Dundee, UK.

FEENEY, D.F., CRAWFORD, J.W., DANIELL, T., HALLETT, P.D., NUNAN, N., RITZ, K., RIVERS, M. AND YOUNG, I.M. (2006a) Three-dimensional microorganization of the soil-root-microbe system. *Microbial Ecology*, **52**, 151-158.

FEENEY, D.F., HALLETT, P.D., RODGER, S., BENGOUGH, A.G., WHITE, N.A. AND YOUNG, I.M. (2006b) Impact of fungal and bacterial biocides on microbial induced water repellency in arable soil. *Geoderma*, **135**, 72-80.

FELSKE, A., WOLTERINK, A., VAN LIS, R., DE VOS, W.M. AND AKKERMANS, A.D.L. (1999) Searching for predominant soil bacteria: 16S rDNA cloning versus strain cultivation. *FEMS Microbiology Ecology*, **30**, 137-145.

FIERER, N. AND SCHIMEL, P. (2002) Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry*, **34**, 777-787.

FIERER, N., SCHIMEL, J.P. AND HOLDEN, P.A. (2003) Influence of drying-rewetting frequency on soil bacterial community structure. *Microbial Ecology*, **45**, 63-71.

FILHO, S. DE P.V., FEIGL, B.J., PICCOLO, M. DE C., FANTE JR. L., NETO, M.S. AND CERRI, C.C. (2004) Root systems and soil microbial biomass under no-tillage system. *Scientia Agricola*, **61**, 529-537.

FILION, M., ST-ARNAUD, M. AND FORTIN, J.A. (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. *New Phytologist*, **141**, 525-533.

FINLAY, R.D. (2004) Mycorrhizal fungi and their multifunctional roles. *Mycologist*, **18**, 91-96.

FITTER, A.H. (2005) Darkness visible: reflections on underground ecology. *Journal of Ecology*, **93**, 231-243.

FITTER, A.H. AND GARBAYE, J. (1994) Interactions between mycorrhizal fungi and other soil organisms. *Plant and Soil*, **159**, 123-132.

FITTER, A.H., GILLIGAN, C.A., HOLLINGWORTH, K., KLECZKOWSKI, A., TWYMAN, R.M., PITCHFORD, J.W. AND THE MEMBERS OF THE NERC SOIL BIODIVERSITY PROGRAMME. (2005) Biodiversity and ecosystem function in soil. *Functional Ecology*, **19**, 369-377.

FITZPATRICK, E.A. (1986) An introduction to soil science. Second edition. Longman Scientific & Technical, UK. Pp255.

FOSTER, R.C. (1988) Microenvironments of soil micro-organisms. *Biology and Fertility of Soils*, **6**, 189-203.

FRACCHIA, S., MUJICA, M.T., GARCÍA-ROMERA, I., GARCÍA-GARRIDO, J.M., MARTÍN, J., OCAMPO, J.A. AND GODEAS, A. (1998) Interactions between *Glomus mosseae* and arbuscular mycorrhizal sporocarp-associated saprophytic fungi. *Plant and Soil*, **200**, 131-137.

GANGE, A.C., BOWER, E., STAGG, P.G., APLIN, D.M., GILLAM, A.E. AND BRACKEN, M. (1999) A comparison of visualization techniques for recording arbuscular mycorrhizal colonisation. *New Phytologist*, **142**, 123-132.

GANGE, A.C., BROWN, V.K. AND FARMER, L.M. (1990) A test of mycorrhizal benefit in an early successional plant community. *New Phytologist*, **115**, 85-91.

GANS, J., WOLINSKY, M. AND DUNBAR, J. (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science*, **309**, 1387-1390.

GARBAYE, J. (1994) Helper bacteria: A new dimension to the mycorrhizal symbiosis. *New Phytologist*, **128**, 197-210.

GARLAND, J.L. (1996) Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilisation. *Soil Biology and Biochemistry*, **28**, 213-221.

GARLAND, J.L. (1997) Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiology Ecology*, **24**, 289-300.

GARLAND, J.L. AND MILLS, A.L. (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology*, **57**, 2351-2359.

GERDEMANN, J.W. (1975) Vesicular-arbuscular mycorrhizae. *In*: TORREY, J.G. AND CLARKSON, D.T. (EDS.). The development and function of roots. Academic Press, London, p 575-591.

GILLER, K.E., BEARE, M.H., LAVELLE, P., IZAC, A.-M.N. AND SWIFT, M.J. (1997) Agricultural intensification, soil biodiversity and agroecosystem function. *Applied Soil Ecology*, **6**, 3-16.

GIRVAN, M.S., BULLIMORE, J., PRETTY, J.N., OSBORN, A.M. AND BALL, A.S. (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soil. *Applied and Environmental Microbiology*, **69**, 1800-1809.

GLIMM E., HEUER, H., ENGELEN, B. SMALLA, K. AND BACKHAUS, H. (1997) Statistical comparisons of community catabolic profiles. *Journal of Microbiological Methods,* **30**, 71–80.

GOMEZ, E.D., GARLAND, J.L. AND ROBERTS, M.S. (2004) Microbial structural diversity estimated by dilution-extinction of phenotypic traits and T-RFLP analysis along a land-use intensification gradient. *FEMS Microbiology Ecology*, **49**, 253-259.

GORDON, D.C. AND HALLETT, P.D. (2009). Rise in CO_2 affects soil water transport through repellency. *Biologia*, **64 (3)**, 532-535.

GRAHAM, J.H., LEONARD, R.T. AND MENGE, J.A. (1981) Membrane-mediated decrease of root exudation responsible for phosphorus inhabitation of vesicular-arbuscular mycorrhiza formation. *Plant Physiology*, **6**, 548-552.

GRAYSTON, S.J., GRIFFITH, G., MAWDSLEY, J.L., CAMPBELL, C.D. AND BARDGETT, R.D. (2001) Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biology and Biochemistry*, **33**, 533-551.

GRAYSTON, S.J., WANG, S., CAMPBELL, C.D. AND EDWARDS, A.C. (1998) Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry*, **30**, 369-378.

GREEN, D.C., VILARIÑO, A., NEWSAM, R., JEFFRIES, P. AND DODD, J.C. (1994) Quantification of mycelial development of arbuscular mycorrhizal fungi using image analysis. *Mycorrhiza*, **5**, 105–113.

GREGORY, P.J. (2006) Plant Roots. Blackwell Publishing Ltd, UK. Pp 318.

GREGORY, P.J., HUTCHISON, D.J., READ, D.B., JENNESON, P.M., GILBOY, W.B. AND MORTON, E.J. (2003) Non-invasive imaging of roots with high resolution X-ray micro-tomography. *Plant Soil,* **255**, 351–359.

GRIFFITHS, B.S., RITZ, K., BARDGETT, R.D., COOK, R., CHRISTENSEN, S., EKELUND, F., SORENSEN, S.J., BÅÅTH, E., BLOEM, J., DE RUITER, P.C., DOLFING, J. AND NICOLARDOT, B. (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity–ecosystem function relationship. *Oikos*, **90**, 279-294.

GRIFFITHS, B.S., RITZ, K., EBBLEWHITE, N. AND DOBSON, G. (1999). Soil microbial community structure: Effects of substrate loading rates. *Soil Biology and Biochemistry*, **31**, 145-153.

GRIFFITHS, B.S., RITZ, K., WHEATLEY, R., KUAN, H.L., BOAG, B., CHRISTENSEN, S., EKELUND, F., SØRENSEN, S.J., MULLER, S. AND BLOEM, J. (2001) An examination of the biodiversity – ecosystem function relationship in arable soil microbial communities. *Soil Biology and Biochemistry*, **33**, 1713 – 1722.

GROFFMAN, P.M., EAGEN, P., SULLIVAN, W.M. AND LEMUNYON, J.L. (1996) Grass species and soil type effects on microbial biomass and activity. *Plant and Soil*, **183**, 61-67.

HAACK, S.K., GARCHOW, H., KLUG, M.J. AND FORNEY, L. (1995) Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilisation patterns. *Applied and Environmental Microbiology*, **61**, 1458-1468.

HABTE, M. AND MANJUNATH, A. (1987) Soil solution phosphorus status and mycorrhizal dependency in *Leucaena leucocephala. Applied and Environmental Microbiology*, **53**, 797-801.

HALL, D.G.M., REEVE, M.J., THOMASSON, A.I. AND WRIGHT, V.F. (1977) Water retention, porosity and density of field soils. Soil Survey Technical Monograph 9. Rothamsted experimental station, Lawes Agricultural Trust, Harpenden, UK.

HALLETT, P.D, BAUMGARTL, T. AND YOUNG, I.M. (2001) Subcritical water repellency of aggregates from a range of soil management practices. *Soil Science Society of America Journal*, **65**, 184-190.

HALLETT, P.D., FEENEY, D.S., BENGOUGH, A.G., RILLIG, M.C., SCRIMGEOUR, C.M. AND YOUNG, I.M. (2009) Disentangling the impact of AM fungi versus roots on soil structure and water transport. *Plant and Soil*, **314**, 183-196.

HALLETT, P.D., GORDON, D.C. AND BENGOUGH, A.G. (2003) Plant influence on rhizosphere hydraulic properties: Direct measurements using a miniaturized infiltrometer. *New Phytologist*, **157**, 597-603.

HALLETT, P.D. AND YOUNG, I.M. (1999) Changes to water repellence of soil aggregates caused by substrate-induced microbial activity. *European Journal of Soil Science*, **50**, 35-40.

HAMZA, M.A., ANDERSON, S.H. AND AYLMORE, L.A.G. (2001) Studies of soil water drawdowns by single radish roots at decreasing soil water content using computer-assisted tomography. *Australian Journal of Soil Research,* **39,** 1387–1396.

HARRIS, K., CRABB, D., YOUNG, I.M., WEAVER, H., GILLIGAN, C.A., OTTEN, W. AND RITZ, K. (2002) *In situ* visualization of fungi in soil thin sections: problems with crystallisation of the fluorochrome FB 28 (Calcofluor M2R) and improved staining by SCRI Renaissance 2200. *Mycological Research*, **106**, 293-297.

HART, M.M. AND READER, R.J. (2002) Taxonomic basis for variation in the colonization strategy for arbuscular mycorrhizal fungi. *New Phytologist*, **153**, 335-344.

HART, M.M., READER, R.J. AND KLIRONOMOS, J.N. (2001) Life-history strategies of arbuscular mycorrhizal fungi in relation to their successional dynamics. *Mycologia*, **93**, 1186-1194.

HARTMANN, A., GIRAUD, J.J. AND CATROUX, G. (1998) Genotypic diversity of *Sinorhizobium* (formly *Rhizobium*) *meliloti* strains isolated directly from a soil and from nodules of alfalfa (*Medicago sativa*) grown in the same soil. *FEMS Microbiology Ecology*, **25**, 107-116.

HARTNETT, D.C. AND WILSON, G.W.T. (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, **80**, 1187-1195.

HATTORI, T. (1988) Soil aggregates as microhabitats of microorganisms. *Report of the Institute of Agricultural Research, Tohoku University*, **37**, 29-36.

HATTORI, T. AND HATTORI, R. (1976) The physical environment in soil microbiology: an attempt to extend principles of microbiology to soil micro-organisms. *Critical Reviews in Microbiology*, **4**, 423-461.

HAYNES, R.J. AND BEARE, M.H. (1996) Aggregation and organic matter storage in meso-thermal, humid soils. *In*: CARTER, M.R. AND STEWARD, B.A. (EDS.) Advances in soil science. Structure and organic matter storage in agricultural soils. CRC Press, Boca Raton, p 213-262.

HAYNES, R.J. AND BEARE, M.H. (1997) Influence of six crop species on aggregate stability and some labile organic matter fractions. *Soil Biology and Biochemistry*, **29**, 1647-1653.

HEDLEY, M.J. AND STEWART, J.W. (1982) Method to measure microbial phosphate in soils. *Soil Biology and Biochemistry*, **14**, 377-385.

HEIJNEN, C.E., HOK-A HIN, C.H. AND VAN VEEN, J.A. (1991) Protection of *Rhizobium* by bentonite clay against predation by flagellates in liquid cultures. *FEMS Microbiology Ecology*, **85**, 65-72.

HEIJS, A.W.J., RITSEMA, C.J. AND DEKKER, L.W. (1996) Three-dimensional visualization of preferential flow patterns in two soils. *Geoderma*, **70**, 101-116.

HELGASON, T., MERRYWEATHER, J.W., DENISON, J., WILSON, P., YOUNG, J.P.W. AND FITTER, A.H. (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. *Journal of Ecology*, **90**, 371-384.

HETTIARATCHI, D.H.P. (1990) Soil compaction and plant root growth. *Philosophical Transactions of the Royal Society of London B*, **329**, 343-355.

HILTNER, L. (1904) Über neuere Erfahrungen und Probleme auf dem Gebiete der Bodenbakteriologie unter besonderer berücksichtigung der Gründüngung und Brache. *Arbiten der Deutschen Landwirtschaftlichen Gesellschaft*, **98**, 59-78. Referenced by:

GREGORY P. (2006) Plant roots: Growth, activity and interaction with soils. Blackwell Publishing, UK. Pp 318.

HINSINGER, P., BENGOUGH, A.G., VETTERLEIN, D. AND YOUNG, I.M. (2009) Rhizopshere: biophysics, biogeochemistry and ecological relevance. *Plant and Soil*, **321**, 117-152.

HODGE, A., ROBINSON, D. AND FITTER, A.H. (2000) An arbuscular mycorrhizal inoculums enhances root proliferation in, but not nitrogen capture from, nutrient-rich patches in soil. *New Phytologist*, **145**, 575-584.

HODGETTS, J., BALL, T., BOONHAM, N., MUMFORD, R. AND DICKINSON, M. (2007) Use of terminal restriction fragment length polymorphism (T-RFLP) for identification of phytoplasmas in plants. *Plant Pathology*, **56**, 357-365.

HORCHANI, F., GALLUSCI, P., BALDET, P., CABASSON, C., MAUCOURT, M., ROLIN, D., ASCHI-SMITI, S. AND RAYMOND, P. (2008) Prolonged root hypoxia induces ammonium accumulation and decreases the nutrientional quality of tomato fruits. *Journal of Plant Physiology*, **165**, 1352-1359.

HORGAN, G.W. (1998) Mathematical morphology for analysing soil structure from images. *European Journal of Soil Science*, **49**, 161-173.

HUNT, H.W. AND WALL, D.H. (2002) Modelling the effects of loss of soil biodiversity on ecosystem function. *Global Change Biology*, **8**, 33-50.

JAEGER III, C.H., LINDOW, S.R., MILLER, W., CLARK, E. AND FIRESTONE, M.K. (1999) Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and trytophan. *Applied and Environmental Microbiology*, **65**, 2685-2690.

JAKOBSEN, I., ABBOTT, L.K. AND ROBSON, A.D. (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum L*. I. Spread of hyphae and phosphorus inflow into roots. *New Phytologist*, **120**, 371–380.

JAKOBSEN, I. AND ROSENDAHL, L. (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist*, **115**, 77-83.

JANSA, J., MOZAFAR, A. AND FROSSARD, E. (2005) Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site. *Plant and Soil*, **276**, 163–176.

JASTROW, J.D. (1996) Soil aggregate formation and the accrual of particulate and mineral-associated organic matter. *Soil Biology and Biochemistry*, **28**, 665-676.

JASTROW, J.D. AND MILLER, R.M. (1991) Assessment of the effects of the biota on soil structure: Methods for assessing the effects of biota on soil structure. *Agriculture, Ecosystems and Environment*, **34**, 279-303.

JASTROW, J.D., MILLER, R.M. AND LUSSENHOP, J. (1998) Contributions of interacting biological mechanisms to soil aggregate stabilisation in restored prairie. *Soil Biology and Biochemistry*, **30**, 905-916.

JENKINSON, D.S. (1966) Studies on the decomposition of plant material in soil. II. Partial sterilization of soil and the soil biomass. *Journal of Soil Science*, **17**, 280-320.

JENKINSON, D.S. (1976) The effects of biocidal treatments on metabolism in soil – IV. The decomposition of fumigated organisms in soil. *Soil Biology and Biochemistry*, **8**, 203-208.

JENKINSON, D.S., BROOKES, P.C. AND POWLSON, D.S. (2004) Measuring soil microbial biomass. *Soil Biology and Biochemistry*, **36**, 5-7.

JENKINSON, D.S. AND POWLSON, D.S. (1976a) The effects of biocial treatments on metabolism in soil – I. Fumigation with chloroform. *Soil Biology and Biochemistry*, **8**, 167-177.

JENKINSON, D.S. AND POWLSON, D.S. (1976b) The effects of biocidal treatments on metabolism in soil – V. A method for measuring soil biomass. *Soil Biology and Biochemistry*, **8**, 209-213.

JENNESON, P.M., GILBOY, W.B., MORTON, E.J. AND GREGORY, P.J. (2003) An X-ray micro-tomography system optimized for the low-dose study of living organisms. *Applied Radiation and Isotopes*, **58**, 177-181.

JOHANSSON, J.F., PAUL, L.R. AND FINLAY, R.D. (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiology Ecology*, **48**, 1-13.

JOHNSON, D., VANDENKOORNHUYSE, P.J., LEAKE, J.R., GILBERT, L., BOOTH, R.E., GRIME, J.P., YOUNG, P.W. AND READ, D.J. (2003) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytologist*, **161**, 503-515.

JOHNSON, N.C., GRAHAM, J.H. AND SMITH, F.A. (1997) Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist*, **135**, 575-585.

JOHNSON, S.N., READ, D.B. AND GREGORY, P.J. (2004) Tracking larval insect movement within soil using high resolution X-ray microtomography. *Ecological Entomology*, **29**, 117-122.

JOSCHKO, M., GRAFF, O., MÜLLER, K., LINDNER, P., PRETSCHNER, D.P. AND LARINK, O. (1991) A non-destructive method for the morphological assessment of earthworm burrow systems in three dimensions by X-ray computed tomography. *Biology and Fertility of Soils,* **11**, 82-92.

JOSCHKO, M., MUELLER, P.C., KOTZKE, K., DOEHRING, W. AND LARINK, O. (1993) Earthworm burrow system development assessed by means of X-ray computed tomography. *Geoderma*, **56**, 209-221.

JOSEPH, S., HUGENHOLTZ, P., SANGWAN, P., OSBORNE, C.A., AND JANSSEN, P.H. (2003) Laboratory cultivation of widespread and previously uncultured soil bacteria. *Applied and Environmental Microbiology*, **69 (12)**, 7210-7215.

KAK, A.C. AND SLANEY, N. (1988) Principles of computerised tomographic imaging. *Society of Industrial and Applied Mathematics*. Available online at: http://www.slaney.org/pct/pct-toc.html (Viewed: 23/07/09).

KAMPICHLER, C. AND HAUSER, M. (1993) Roughness of soil pore surface and its effect on available habitat space of microarthropods. *Geoderma*, **56**, 223-232.

KARLEN, D.L., MAUSBACH, M.J., DORAN, J.W., CLINE, R.G., HARRIS, R.F. AND SCHUMAN, G.E. (1997) Soil quality: A concept, definition, and framework for evaluation (A guest editorial). *Soil Science Society of America Journal*, **61**, 4-10.

KEMPER, W.D. AND KOCH, E.J. (1966) Aggregate stability of soils from western United States and Canada. *In: USDA-ARS Technical Bulletin*, **1355**, U.S. Government print office, Washington, D.C.

KEMPER, W.D. AND ROSENAU, R.C. (1986) Aggregate stability and size distribution, p 425-442. *In:* KLUTE, A (ED.) Methods of soil analysis. Part 1: Physical and mineralogical methods. 2nd ed. American Society of Agronomy, Madison, WI.

KENNEDY, N., BRODIE, E., CONNOLLY, J. AND CLIPSON, N. (2004) Impact of lime, nitrogen and plant species on bacterial community structure in grassland microcosms. *Environmental Microbiology*, **6**, 1070-1080.

KENNEDY, A.C. AND SMITH, K.L. (1995) Soil microbial diversity and the sustainability of agricultural soils. *Plant and Soil*, **170**, 75-86.

KÉZDI, A. (1974) Handbook of soil mechanics. Vol. 1: Soil Physics. Elsevier Scientific Publishing, London. Pp 34.

KIEM, R. AND KANDELER, E. (1997) Stabilisation of aggregates by the microbial biomass as affected by soil texture and type. *Applied Soil Ecology*, **5**, 221-230.

KIRK, J.L., BEAUDETTE, L.A., HART, M., MOUTOGLIS, P., KLIRONOMOS, J.N., LEE, H. AND TREVORS, J.T. (2004) Methods of studying soil microbial diversity. *Journal of Microbiological Methods*, **58**, 169-188.

KLARING, H.P. AND ZUDE, M. (2009) Sensing of tomato plant response to hypoxia in the root environment. *Scientia Horticulturae*, **22**, 17-25.

KLEINFELDER, D., SWANSON, S., NORRIS, G. AND CLARY, W. (1992) Unconfined compressive strength of some streambank soils with herbaceous roots. *Soil Science Society of America Journal*, **56**, 1920-1925.

KLIRONOMOS, J.N. (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, **84**, 2292-2301.

KLIRONOMOS, J.N., ALLEN, M.F., RILLIG, M.C., PIOTROWSKI, J., MAKVANDI-NEJAD, S., WOLFE, B.E. AND POWELL, J.R. (2005) Abrupt rise in atmospheric CO_2 overestimates community response in a model plant-soil system. *Nature*, **433**, 621-624.

KLIRONOMOS, J.N., MCCUNE, J., HART, M. AND NEVILLE, J. (2000) The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecology Letters*, **3**, 137-141.

KLIRONOMOS, J.N., MCCUNE, J. AND MOUTOGLIS, P. (2004) Species of arbuscular mycorrhizal fungi affect mycorrhizal responses to simulated herbivory. *Applied Soil Ecology*, **26**, 133-141.

KLIRONOMOS, J.N. AND URSIC, M. (1998). Density-dependent grazing on the extraradical hyphal network of the arbuscular mycorrhizal fungus *Glomus intraradices* by the collembolan *Folsomia candida*. *Biology and Fertility of Soils,* **26**, 250-253.

KONOPKA, A., OLIVER, L. AND TURCO, R.F. (1998) The use of carbon substrate utilization patterns in environmental and ecological microbiology. *Microbial Ecology,* **35**, 103–115.

KOOISTRA, M.J., SCHOONDERBEEK, D., BOONE, F.R., VEEN, B.W. AND VAN NOORDWIJK, M. (1992) Root-soil contact of maize, as measured by thin-section technique. *Plant and Soil*, **139**, 119-129.

KORMANIK, P.P. AND MCGRAW, A.-C. (1982) Quantification of vesicular-arbuscular mycorrhizae in plant roots. *In*: SCHENK, N.C. (ED.) Methods and principles of mycorrhizal research. St Paul, Minnesota, USA. American Phytopathological Society, p 37-45.

KOSKE, R.E. AND GEMMA, J.N. (1989) A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research,* **92**, 486–488.

KOSKE, R.E., GEMMA, J.N. AND FLYNN, T. (1992) Mycorrhizae in Hawaiian angiosperms: a survey with implications for the origin of the native flora**.** *American Journal of Botany*, **79,** 853-862.

KRISHNA, K.R. AND BAGYARAJ, D.J. (1982) Effect of vesicular arbuscular mycorrhiza and soluble phosphate on *Abelmoscus esculentus (L.). Plant and Soil,* **64**, 209-213.

KUIKMAN, P.J., VAN VUUREN, M.M.I. AND VAN VEEN, J.A. (1989) Effect of soil moisture on the predation by protozoa of bacteria biomass and the release of bacterial nitrogen. *Agricultural Ecosystem Environment*, **27**, 271-279.

LAAN, P., CLEMENT, J.M.A.M. AND BLOM, C.W.P.M. (1991) Growth and development of *rumex* roots as affected by hypoxic and anoxic conditions. *Plant and Soil*, **136**, 145-151.

LANGMAACK, M., SCHRADER, S., RAPP-BERNHARDT, U. AND KOTZKE, K. (2002) Soil structure rehabilitation of arable soil degraded by compaction. *Geoderma*, **105**, 141-152.

LATOUR, X., CORBERAND, T., LAGUERRE, G., ALLARD, F. AND LEMANCEAU, P. (1996) The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. *Applied and Environmental Microbiology*, **62**, 2449-2456.

LE BISSONNIAS, Y. (1996) Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. *European Journal of Soil Science*, **47**, 425-437.

LEEDS-HARRISON, P.B. AND YOUNGS, E.G. (1997) Estimating the hydraulic conductivity of aggregates conditioned by different tillage treatments from sorption measurements. *Soil Tillage Research*, **41**, 141-147.

LEEDS-HARRISON, P.B., YOUNGS, E.G. AND UDDIN, B. (1994) A device for determining the sorptivity of soil aggregates. *European Journal of Soil Science*, **45**, 269-272.

LEIJ, F.J., GHEZZEHEI, T.A. AND OR, D. (2002) Analytical models for soil pore-size distributions after tillage. *Soil Science Society of America Journal*, **66**, 1104-1114.

LIGTHART, T.N. (1997) Thin section analysis of earthworm burrow disintegration in a permanent pasture. *Geoderma*, **75**, 135-148.

LIN, Q. AND BROOKES, P.C. (1999) An evaluation of the substrate-induced respiration method. *Soil Biology and Biochemistry*, **31**, 1969-1983.

LINDERMAN, R.G. (1997) Vesicular-arbuscular mycorrhizal (VAM) fungi, p 117-128. *In*: CARROLL, G.C. AND TUDZYNSKI, P. (EDS.) The mycota. Springer-Verlag, Berlin, Germany.

LINN, D.M. AND DORAN, J.W. (1984) Aerobic and anaerobic microbial populations in no-till and ploughed soils. *Soil Science Society of America Journal.* **48**, 794–799.

LIU, B.-R., JIA, G.-M., CHEN, J. AND WANG, G. (2006) A review of methods for studying microbial diversity in soils. *Pedosphere*, **16**, 18-24.

LIU, W.T., MARSH, T.L., CHENG, H. AND FORNEY, L.J. (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphorism of genes encoding 16S rRNA. *Applied and Environmental Microbiology*, **63**, 4516-4522.

LONTOC-ROY, M., DUTILLEUL, P., PRASHER, S.O., HAN, L., BROUILLET, T. AND SMITH, D.L. (2006) Advances in the acquisition and analysis of CT scan data to isolate a crop root system from the soil medium and quantify root system complexity in 3-D space. *Geoderma*, **137**, 231-241.

LORD, N.S., KAPLAN, C., SHANK, P., KITTS, C.L. AND ELROD, S.L. (2002) Assessment of fungal diversity using terminal restriction fragment (T-RF) pattern analysis: comparison of 18S and ITS ribosomal regions. *FEMS Microbiology Ecology*, **42**, 327-337.

LOREAU, M., NAEEM, S., INCHAUSTI, P., BENGTSSON, J., GRIME, J.P., HECTOR, A., HOOPER, D.U., HUSTON, M.A., RAFFAELLI, D., SCHMID, B., TILMAN, D. AND WARDLE, D.A. (2001) Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science*, **26**, 804-808.

LUKOW, T., DUNFIELD, P.F. AND LIESACK, W. (2000) Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-trangenic potato plants. *FEMS Microbiology Ecology*, **32**, 241-247.

LUO, L., LIN, H. AND HALLECK, P. (2008) Quantifying soil structure and preferential flow in intact soil using X-ray computed tomography. *Soil Science Society of America*, **72**, 1058-1069.

LUPWAYI, N.Z., ARSHAD, M.A., RICE, W.A. AND CLAYTON, G.W. (2001) Bacterial diversity in water-stable aggregates of soil under conventional and zero tillage management. *Applied Soil Ecology*, **16**, 251-261.

LYNCH, J.M. AND BRAGG, E. (1985) Microorganisms and soil aggregate stability. *Advances in Soil Science*, **2**, 133-171.

LYNCH, J.M. AND WHIPPS, J.M. (1990) Substrate flow in the rhizosphere. *Plant and Soil*, **129**, 1-10.

MACEDO, A., VAZ, C.M.P., NAIME, J.M., CRUVINEL, P.E., CRESTANA, S. (1999) X-ray microtomography to characterize the physical properties of soil and particulate system. *Powder Technology,* **101**, 178-182.

MAGID, J., KJÆRGAARD, C., GORISSEN, A. AND KUIKMAN, P.J. (1999) Drying and rewetting of a loamy sand soil did not increase the turnover of native organic matter, but retarded the decomposition of added ^{14}C-labelled plant material. *Soil Biology and Biochemistry*, **31**, 595-602.

MARSCHNER, H. (1995) Mineral nutrition of higher plants. 2nd Edition. Academic Press, London. Pp869.

MARSCHNER, P. AND BAUMANN, K. (2003) Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. *Plant and Soil*, **251**, 279-289.

MARSH, T.L., SAXMAN, P., COLE, J. AND TIEDJE, J. (2000) Terminal restriction fragment length polymorphorism analysis orogram, a web-based research tool for microbial community analysis. *Applied and Environmental Microbiology*, **66**, 3616-3620.

MCGONIGLE, T.P., MILLER, M.H., EVANS, D.G., FAIRCHILD, G.L. AND SWAN, J.A. (1990) A new method which gives an objective measure of colonisation of root by vesicular arbuscular mycorrhizal fungi. *New Phytologist,* **115**, 495-501.

MEES, F., SWENNEN, R., VAN GEET, M. AND JACOBS, P. (2003) Applications of X-ray computed tomography in the geosciences. *Geological Society, London, Special Publications*, **215**, 1-6.

MENGE, J.A., JOHNSON, E.L.V. AND PLATT, R.G. (1978) Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. *New Phytologist*, **81**, 553-559.

MERMUT, A.R., GREVERS, M.C.J. AND DE JONG, E. (1992) Evaluation of pores under different management systems by image analysis of clay soils in Saskatchewan, Canada. *Geoderma*, **53**, 357–372.

MILLER, R.M. AND JASTROW, J.D. (1990) Hierarchy of roots and mycorrhizal fungal interactions with soil aggregation. *Soil Biology and Biochemistry*, **22 (5)**, 579-584.

MILNE, R.M. AND HAYNES, R.J. (2004) Soil organic matter, microbial properties, and aggregate stability under annual and perennial pastures. *Biology of Fertile Soils*, **39**, 172-178.

MOHAMED, A.M.O. AND ANITA, H.E. (1998) Geoenvironmental engineering. *Developments in Geotechnical Engineering*, **82**. Elsevier, UK. Pp 707.

MOONEY, S.J. (2002) Three-dimensional visualization and quantification of soil macroporosity and water flow patterns using computed tomography. *Soil Use and Management*, **18**, 142-151.

MOONEY, S.J., MORRIS, C., CRAIGON, J. AND BERRY, P. (2007) Quantification of soil structural changes induced by cereal anchorage failure: Image analysis of thin sections. *Journal of Plant Nutrition and Soil Science*, **170**, 363-372.

MORAN, C.J., KOPPI, A.J., MURPHY, B.W. AND MCBRATNEY, A.B. (1988) Comparison of the macropore structure of a sandy loam surface soil horizon subjected to two tillage treatments. *Soil Use and Management*, **4**, 96-102.

MOREL, J.L., HABIB, L., PLANTUREUX, S. AND GUCKERT, A. (1991) Influence of maize root mucilage on soil aggregate stability. *Plant and Soil*, **136**, 111-119.

MORENO-ESPÍNDOLA, I.P., RIVERA-BECERRIL, F., FERRARA-GUERRERO, M.J. AND DE LÉON-GONZÁLEZ, F. (2007) Role of root-hairs and hyphae in adhesion of sand particles. *Soil Biology and Biochemistry*, **39**, 2520-2526.

MORIN, F., FORTIN, J.A., HAMEL, C., GRANGER, R.L. AND SMITH, D.L. (1994) Apple rootstock response to vesicular-arbuscular mycorrhizal fungi in a high phosphorous soil. *Journal of the American Society of Horticultural Science*, **119**, 578-583.

MOSSE, B. (1972) The influence of soil type and *Endogone* strain on the growth of mycorrhizal plants in phosphate-deficient soils. *European Journal of Soil Biology*, **9**, 529-537.

MOSSE, B., HAYMAN, D.S. AND ARNOLD, D.J. (1973) Plant growth response to vesicular arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with ^{32}P. *New Phytologist,* **72**, 809–815.

MTAMBANENGWE, F., MAPTUMO, P. AND KIRCHMANN, H. (2004) Decomposition of organic matter in soil as influenced by texture and pore size. *In:* BATIONO, A. (ED.) Managing nutrient cycles to sustain soil fertility in Sub-Saharan Africa. Academy Science Publishers, a Division of the African Academy of Sciences (AAS), Nairobi. Available at: http://www.ciat.cgiar.org/tsbf_institute/book_mgmt_nutri_cycles.htm?codigo=P324. (Viewed: 25/03/09).

MULLA, D.J., HUYCK, L.M. AND REGANOLD, J.P. (1992) Temporal variations in aggregate stability on conventional and alternative farms. *Soil Science Society of America Journal*, **56**, 1620-1624.

MUNKVOLD, L., KJOLLER, R., VESTBERG, M., ROSENDAHL, S. AND JAKOBSEN, I. (2004) High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist*, **164**, 357–364.

NAEEM, S., LOREAU, M. AND INCHAUSTI, P. (2002) Biodiversity and ecosystem function: the emergence of a synthetic ecological framework, p 3-11. *In*: LOREAU, M., NAEEM, S. AND INCHAUSTI, P. (EDS.) (2002) Biodiversity and ecosystem functioning: synthesis and perspectives. Oxford University Press, UK. Pp294.

NAEEM, S., THOMPSON, L.J., LAWLER, S.P., LAWTON, J.H. AND WOODFIN, R.M. (1994) Declining biodiversity can alter the performance of ecosystems. *Nature*, **368**, 734-737.

NANNIPIERI, P., ASCHER, J., CECCHERINI, M.T., LANDI, L., PIETRAMELLARA, G. AND RENELLA, G. (2003) Microbial diversity and soil function. *European Journal of Soil Science*, **54**, 655-670.

NANNIPIERI, P., ASCHER, J., CECCHERINI, M.T., LANDI, L., PIETRAMELLARA, G., RENELLA, G. AND VALORI, F. (2008) Effects of root exudates in microbial diversity and activity in rhizosphere soils. *Soil Biology*, **15**, 339-365.

NATIONAL RESEARCH COUNCIL (1993) Soil and water quality: an agenda for agriculture. National Academies Press. Pp 516.

NEINHUIS, C. AND BARTHLOTT, W. (1997) Characterisation and distribution of water repellent, self-cleaning plant surfaces. *Annals of Botany,* **79**, 667–677.

NEWHAM, E.I. AND REDDELL, P. (1987) The distribution of mycorrhizas among families of vascular plants. *New Phytologist*, **106**, 745-751.

NEWSHAM, K.K., FITTER, A.H. AND WATKINSON, A.R. (1995a) Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology*, **83**, 991-1000.

NEWSHAM, K.K., FITTER, A.H. AND WATKINSON, A.R. (1995b) Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology and Evolution*, **10**, 407-411.

NISHIO, M. AND FURUSAKA, C. (1970) The distribution of nitrifying bacteria in soil aggregates. *Soil Science and Plant Nutrition*, **16**, 24-29.

NOGUCHI, S., NIK, A.R., KASRAN, B., TANI, M., SAMMORI, T. AND MORISADA, K. (1997) Soil physical properties and preferential flow pathways in tropical rain forest, Bukit Tarek, Peninsular Malaysia. *Journal of Forest Research*, **2**, 115-120.

NUNAN, N., DANIELL, T.J., SINGH, B.K., PAPERT, A., MCNICOL, J.W. AND PROSSER, J.I. (2005) Links between plant and rhizoplane bacterial communities in grassland soils, characterized using molecular techniques. *Applied and Environmental Microbiology*, **71**, 6784-6792.

NUNAN, N., RITZ, K., CRABB, D., HARRIS, K., WU, K., CRAWFORD, J.W. AND YOUNG, I.M. (2001) Quantification of the in situ distribution of soil bacteria by large-scale imaging of thin sections of undisturbed soil. *FEMS Microbiology Ecology*, **36**, 67-77.

NUNAN, N., RITZ, K., RIVERS, M., FEENEY, D.S. AND YOUNG, I.M. (2006) Investigating microbial micro-habitat structure using X-ray computed tomography. *Geoderma*, **133**, 398-407.

NUNAN, N., WU, K., YOUNG, I.M., CRAWFORD, J.W. AND RITZ, K. (2002) *In Situ* spatial patterns of soil bacterial populations, mapped at multiple scales, in an arable soil. *Microbial Ecology*, **44**, 296-305.

NYE, P.H. AND TINKER, P.B. (1997) Solute movement in the soil-root system, University of California press, Berkeley. Pp 342.

O'DONNELL, A.G., SEASMAN, M., MACRAE, A., WAITE, I. AND DAVIES, J.T. (2001) Plant and fertilisers as drivers of change in microbial community structure and function in soils. *Plant and Soil*, **232**, 135-145.

OADES, J.M. (1984) Soil organic matter and structural stability: mechanisms and implications for management. *Plant and Soil*, **76**, 319-337.

OADES, J.M. (1993) The role of biology in the formation, stabilization and degradation of soil structure. *Geoderma*, **56**, 377-400.

OADES, J.M. AND WATERS, A.G. (1991) Aggregate hierarchy in soils. *Australian Journal of Soil Research*, **29 (6)**, 815-828.

OLDEMAN, L.R. (1998) Soil degradation: A threat to food security? Report 98/01, International Soil Reference and Information Centre (ISRIC), Wageningen.

OLIVERIA, R.S., CASTRO, P.M.L., DODD, J.C. AND VOSÁTKA, M. (2006) Different native arbuscular mycorrhizal fungi influence the coexistence of two plant species in a highly alkaline anthropogenic sediment. *Plant and Soil*, **287**, 209-221.

OTTEN, W., HARRIS, K., YOUNG, I.M., RITZ, K. AND GILLIGAN, C.A. (2004) Preferential spread of the pathogenic fungus *Rhizoctonia solani* through structured soil. *Soil Biology and Biochemistry*, **36**, 203-210.

PACHEPSKY, Y., RAWLS, W. AND TIMLIN, D. (2000). A one-parameter relationship between unsaturated hydraulic conductivity and water retention. *Soil Science*, **165**, 911–919.

PACHEPSKY, Y., YAKOVCHENKO, V., RABENHORST, M.C., POOLEY, C. AND SIKORA, L.J. (1996) Fractal parameters of pore surfaces as derived from micromorphological data*:* Effect of long**-**term management practices. *Geoderma,* **74,** 305–319.

PAGLIAI, M., VIGNOZZI, N. AND PELLEGRINI, S. (2004) Soil structure and the effect of management practices. *Soil and Tillage Research*, **79**, 131-143.

PAPADOPOULOS, A. (2007) Quantifying physical aspects of soil structure associated with organic management practices. PhD thesis, University of Nottingham, UK.

PEPPER, I.L., GERBA, C.P. AND BRENDECKE, J.W. (1995) Environmental microbiology: A laboratory manual. Academic Press Limited, London. Pp 175.

PERRET, J., PRASHER, S.O., KANTZAS, A. AND LANGFORD, C., (1999) Three-dimensional quantification of macropore networks in undisturbed soil cores. *Soil Science of America Journal*, **63**, 1530-1543.

PETROVIC, A.M., SIEBERT, J.E. AND RIEKE, P.E. (1982) Soil bulk density analysis in three dimensions by computer tomography scanning. *Soil Science Society of America*, **46**, 445-450.

PHILIP, J.R. (1957) The theory of infiltration I. The infiltration equation and its solution. *Soil Science*, **83**, 345-357.

PHILIPPOT, L., RENAULT, P., SIERRA, J., HENAULT, C., CLAYS-JOSSERAND, A., CHENU, C., CHAUSSOD, R. AND LENSI, R. (1996) Dissimilatory nitrite-reductase provides a competitive advantage to *Pseudomonas sp.* RTC01 to colonise the centre of soil aggregates. *FEMS Microbiology Ecology*, **21**, 175-185.

PHILIPS, J.M. AND HAYMAN, D.S. (1970) Improved procedure of clearing roots and staining parasitic and VAM fungi for rapid assessment of colonisation. *Transactions of the British Mycological Society,* **55**, 158–161.

PICCOLO, A. AND MBAGWU, J.S.C. (1990) Effects of different organic waste amendments on soil microaggregates stability and molecular size of humic substances. *Plant and Soil*, **123**, 27-37.

PICCOLO, A. AND MBAGWU, J.S.C. (1999) Role of hydrophobic components of soil organic matter in soil aggregate stability. *Soil Science Society of America Journal*, **63**, 1801-1810.

PIERRET, A., CAPOWIEZ, Y., BELZUNCES, L. AND MORAN, C.J. (2002) 3D reconstruction and quantification of macropores using X-ray computed tomography and image analysis. *Geoderma,* **106**, 247-273.

PIERRET, A., CAPOWIEZ, Y., MORAN, C. AND KRETZSCHMAR, A. (1999) X-ray computed tomography to quantify tree rooting spatial distributions. *Geoderma,* **90**, 307–326.

PIMENTAL, D. AND GIAMPIETRO, M. (1994) Food, land, population and the U.S. economy. Executive summary. Carry capacity network. Washington, DC: Available at: http://dieoff.org/page40.htm. (Viewed: 23/07/09).

PIMM, S.L., RUSSELL, G.J., GITTLEMAN, J.L. AND BROOKS, T.M. (1995) The future of biodiversity. *Science,* **269**, 347–350.

PIO, C.A., CASTRO, L.M., CERQUEIRA, M.A., SANTOS, I.M., BELCHIOR, F. AND SALGUEIRO, M.L. (1996) Source assessment of particulate air pollutants measured at the Southwest European coast. *Atmospheric Environment*, **30**, 3309-3320.

PIOTROWSKI, J.S., DENICH, T., KLIRONOMOS, J.N., GRAHAM, J.M. AND RILLIG, M.C. (2004) The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species. *New Phytologist*, **164**, 365-373.

PIRES, L.F., BACCHI, O.O.S. AND REICHARDT, K. (2005) Gamma ray computed tomography to evaluate wetting/drying soil structure changes. *Nuclear Instruments and Methods in Physics Research B*, **229**, 443-456.

PITTY, A. F. (1979) Geography and soil properties. Taylor and Francis, London. Pp 287.

PLANTE, A.F., FENG, Y. AND MCGILL, W.B., (2002) A modelling approach to quantifying soil macroaggregate dynamics. *Canadian Journal of Soil Science*, **82**, 181-190.

PLENCHETTE, C. (1991) Utilisation des mycorhizes en agriculture et horticulture. In: STRULLU, D.G., GARBAYE, J., PERRIN, P. AND PLENCHETTE, C. (EDS.) Les Mycorhizes de Arbes et Plantes Cultivées. Technique et Documentations, Lavoiser, p 131-196.

PLENCHETTE, C., FORTIN, J.A. AND FURLAN, V. (1983) Growth responses to several plant species to mycorrhizae in the soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. *Plant and Soil*, **70**, 199-209.

PLENCHETTE, C., FURLAN, V. AND FORTIN, J.A. (1981) Growth simulation of apple trees in unsterilized soils under field conditions with VA mycorrhizal inoculums. *Canadian Journal of Botany*, **59**, 2003-2008.

PRESTON-MAFHAM, J., BODDY, L. AND RANDERSON, P.F. (2002) Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles – a critique. *FEMS Microbiology Ecology*, **42**, 1-14.

PULLEMAN, M.M., SIX, J., VAN BREEMEN, N. AND JONGMANS, A.G. (2005) Soil organic matter distribution and microaggregate characteristics as affected by agricultural management and earthworm activity. *European Journal of Soil Science*, **56**, 453-467.

RAMETTE, A. (2007) Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology*, **62**, 142-160.

RANDALL, E.W., MAHIEU, N. AND IVANOVA, G.I. (1997) NMR studies of soil, soil organic matter and nutrients: Spectroscopy and imaging. *Geoderma*, **80**, 307-325.

RANJARD, L., RICHAUME, A., JOCTEUR MONROZIER, L. AND NAZARET, S. (1997) Response of soil bacteria to Hg(II) in relation to soil characteristics and cell location. *FEMS Microbiology Ecology*, **24**, 321-331.

RAVNSKOV, S., NYBROE, O. AND JAKOBSEN, I. (1999) Influence of an arbuscular mycorrhizal fungus on *Pseudomonas fluorescens* DF57 in rhizosphere and hyphosphere soil. *New Phytologist,* **142**, 113–122.

READ, D.B., BENGOUGH, A.G., GREGORY, P.J., CRAWFORD, J.W., ROBINSON, D., SCRIMGEOUR, C.M., YOUNG, I.M., ZHANG, K. AND ZHANG, X. (2003) Plant roots release phospholipid

surfactants that modify the physical and chemical properties of soil. *New Phytologist*, **157**, 315-326.

REID, J.B. AND GOSS, M.J. (2006) Effect of living roots of different plant species on the aggregate stability of two arable soils. *European Journal of Soil Science*, **32**, 521-541.

REMENANT, B., GRUNDMANN, G.L. AND JOCTEUR-MONROZIER, L. (2009) From the micro-scale to the habitat: Assessment of soil bacterial community structure as shown by soil structure directed sampling. *Soil Biology & Biochemistry*, **41**, 29-36.

RENGASAMY, P., GREENE, R.S.B., FORD, G.W. AND MEHANNI, A.H. (1984) Identification of dispersive behaviour and the management of red-brown earths. *Australian Journal of Soil Research*, **22**, 413-431.

RILLIG, M.C. (2004) Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science*, **84**, 355-363.

RILLIG, M.C. AND MUMMY, D.L. (2006) Mycorrhizas and soil structure. *New Phytologist*, **171**, 41-53.

RILLIG, M.C., MUMMEY, D.L., RAMSEY, P.W., KLIRONOMOS, J.N. AND GANNON, J.E. (2006) Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. *FEMS Microbiology Ecology,* **57**, 389-395.

RILLIG, M.C., WRIGHT, S.F AND EVINER, V.T. (2002) The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. *Plant and Soil*, **238**, 325-333.

RINGROSE-VOASE, A.J. (1987) A scheme for the quantitative description of soil macrostructure by image analysis. *Journal of Soil Science*, **38**, 343-356.

RINGROSE-VOASE, A.J. (1996) Measurement of soil macropores geometry by image analysis of sections through impregnated soil. *Plant and Soil*, **184**, 27-47.

RINGROSE-VOASE, A.J. AND BULLOCK, P. (1984) the automatic recognition and measurement of soil pore types by image analysis and computer programs. *Journal of Soil Science*, **35**, 673-684.

RITSEMA, C.J., DEKKER, L.W., HENDRICKX, J.M.H. AND HAMMINGA, W. (1993) Preferential flow mechanisms in water repellency sandy soil. *Water Resources Research*, **29**, 2183-2193.

RITZ, K. (2007) The plate debate: Cultivable communities have no utility in contemporary environmental microbial ecology. *FEMS Microbiology Ecology*, **60**, 358-362.

RITZ, K. AND YOUNG, I.M. (2004) Interactions between soil structure and fungi. *Mycologist*, **18**, 52-59.

ROGERS, H.H. AND BOTTOMLEY, P.A. (1987) *In situ* nuclear magnetic resonance imaging of roots: influence of soil type, ferromagnetic particle content and soil water. *Agronomy Journal*, **79**, 957–965.

ROWELL, D.L. (1994) Soil Science: Methods and Applications. Longman Group, UK. Pp350.

RUTHERFORD, P.M. AND JUMA, N.G. (1992) Influence of texture on habitable pore space and bacteria-protozoan populations in soil. *Biology and Fertility of Soil*, **12**, 221-227.

SAGGAR, S., BETTANY, J.R. AND STEWART, J.W.B. (1981) Measurement of microbial sulfur in soil. *Soil Biology and Biochemistry*, **13**, 493-498.

SAIKKONEN, K., AHONEN-JONNARTH, U., MARKKOLA, A.M., HELANDER, M., TUOMI, J., ROITTO, M. AND RANTA, H. (1999) Defoliation and mycorrhizal symbiosis: a functional balance between carbon sources and below-ground sinks. *Ecology Letters*, **2**, 19-26.

SAIT, M., HUGENHOLTZ, P. AND JANSSEN, P.H. (2002) Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. *Environmental Microbiology*, **4**, 654-666.

SAKAI, M., MATSUKA, A., KOMURA, T. AND KANAZAWA, S. (2004) Application of a new primer for terminal restriction fragment length polymorphism analysis of the bacterial communities in plant roots. *Journal of Microbiological Methods*, **59**, 81-89.

SALONIUS, P.O. (1981) Metabolic capabilities of forest soil microbial populations with reduced species diversity. *Soil Biology and Biochemistry*, **13**, 1-10.

SAMPSON, R.N. AND SCHOLES, R.J. (2000) Additional human-induced activities. Article 3.4. p181-281. *In*: WATSON, R.T., NOBLE, I.R., BOLIN, B., RAVINDRANATH, N.H., VERARDO, D.J.

AND DOKKEN, D.J. (EDS.) Land use, land-use change and forestry: A special report of the Intergovernmental Panel on Climate Change. Cambridge University Press. New York.

SCHAPP, M.G. AND LEBRON, I. (2001) Using microscope observations of thin sections to estimate soil permeability with the Kozeny-Carman equation. *Journal of Hydrology*, **251**, 186-201.

SCHJØNNING, P., ELMHOLT, S., MINKHOLM, L. AND DEBOSZ, K. (2002) Soil quality aspects of humid sandy loams influenced by organic and conventional long-term management. *Agriculture, Ecosystems and Environment*, **88**, 195-214.

SCHREINER, R.P., MIHARA, K.L., MCDANIEL, H. AND BETHLENFALVAY, G.J. (1997) Mycorrhizal fungi influence plant and soil functions and interactions. *Plant and Soil*, **188**, 199-209.

SCHUBERT, A. AND LUBRACO, G. (2000) Mycorrhizal inoculation enhances growth and nutrient uptake of micropropagated apple rootstocks during weaning in commercial substrates of high nutrient availability. *Applied Soil Ecology*, **15**, 113-118.

SCULLION, J., NEALE, S. AND PHILIPPS, L. (2002) Comparisons of earthworm populations and cast properties in conventional and organic arable rotations. *Soil Use and Management*, **18**, 293-300.

SIKES, B.A., COTTENIE, K. AND KLIONOMOS, J.N. (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular myccorhizas. *Journal of Ecology*, **97**, 1274-1280.

SIMONS, F.J., VERHELST, F. AND SWENNEN, R. (1997) Quantitative characterization of coal by mean of microfocal X-ray computed microtomography (CMT) and color image analysis (CIA). *International Journal of Coal Geology*, **34**, 69-88.

SINGER, M.J., SOUTHARD, R.J., WARRINGTON, D.N. AND JANITZKY, P. (1992) Stability of synthetic sand-clay aggregates after wetting and drying cycles. *Soil Science Society America Journal*, **56**, 1843-1848.

SIX, J., BOSSUYT, H., DE GRYZ, S. AND DENEF, K. (2004) A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research*, **79**, 7-31.

SIX, J., ELLIOTT, E.T. AND PAUSTIAN, K. (2000) Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry*, **32**, 2099-2103.

SIX, J., ELLIOTT, E.T., PAUSTIAN, K. AND DORAN, J.W. (1998) Aggregation and soil organic matter dynamics under conventional and no-tillage systems. *Soil Science Society of America Journal*, **63**, 1350-1358.

SIX, J., PAUSTIAN, K., ELLIOTT, E.T., AND COMBRINK, C. (2000) Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Science Society American Journal*, **64**, 681-689.

SMALLA, K., WACHTENDORF, U., HEUER, H., LIU, W. AND FORNEY, L. (1998) Analysis of Biolog GN substrate utilization patterns by microbial communities. *Applied and Environmental Microbiology,* **64**, 1220–1225.

ŠMILAUER, P. (2001) Communities of arbuscular mycorrhizal fungi in grassland: Seasonal variability and effects of environment and host plants. *Folia Geobotanica*, **36**, 243-263.

ŠMILAUEROVÁ, M. AND ŠMILAUER, P. (2002) Morphological responses of plant roots to heterogeneity of soil resources. *New Phytologist*, **154**, 703-715.

SMITH, F.A. AND SMITH, S.E. (1997) Tansley Review No. 96: Structural diversity in (vesicular) - arbuscular mycorrhizal symbioses. *New Phytologist*, **137**, 373-388.

SMITH, S.E. AND READ, D.J. (1997) Mycorrhizal Symbiosis. Academic Press, London. Pp787.

SOUTY, N. AND RODE, C. (1993) Emergence of sugar beet seedlings from under different obstacles. *European Journal of Agronomy*, **2**, 213-221.

SPACCINI, R., PICCOLO, A., CONTE, P., HABERHAUSER, G. AND GERZABEK, M.H. (2002) Increased soil organic carbon sequestration through hydrophobic protection by humic substances. *Soil Biology and Biochemistry*, **34**, 1839-1851.

STADDON, P.L. (1998) Insights into mycorrhizal colonisation at elevated CO_2: a simple carbon partitioning model. *Plant and Soil*, **205**, 171-180.

STADDON, P.L., GRAVES, J.D. AND FITTER, A.H. (1998) Effect of enhanced atmospheric CO_2 on mycorrhizal colonisation by *Glomus mosseae* in *Plantago lanceolata* and *Trifolium repens*. *New Phytologist*, **139**, 571-580.

STIRZAKER, R.J., PASSIOURA, J.B. AND WILMS, Y. (1996) Soil Structure and plant growth: Impact of bulk density and biopores. *Plant and Soil*, **185**, 151-162.

SWIFT, M.J., IZAC, A.-M.N. AND VAN NOORDWIJK, M. (2004) Biodiversity and ecosystem services in agricultural landscapes – are we asking the right questions? *Agriculture, Ecosystems and Environment*, **104**, 113-134.

TAINA, I.A., HECK, R.J AND ELLIOT, T.R. (2008) Application of X-ray computed tomography to soil science: A literature review. *Canadian Journal of Soil Science*, **88**, 1-20.

TATE, R.L. (2000) Soil microbiology: Second edition. John Wiley and Sons, Inc., Pp508.

TAWARAYA, K., TOKAIRIN, K. AND WAGATSUMA, T. (2001) Dependence of *Allium fistulosum* cultivars on the arbuscular mycorrhizal fungus, *Glomus fasciculatum*. *Applied Soil Ecology*, **17**, 119-124.

THIEME, J., SCHNEIDER, G. AND KNÖCHEL, C. (2003) X-ray tomography of a microhabitat of bacteria and other soil colloids with sub-100 nm resolution. *Micron*, **34**, 339-344.

THOMSEN, I.K., SCHOJØNNING, P., JENSEN, B., KRISTENSEN, K. AND CHRISTENSEN, B.T. (1999) Turnover of organic matter in differently textured soils II. Microbial activity as influenced by soil water regimes. *Geoderma*, **89**, 199-218.

TIEDJE, J.M., ASUMING-BREMPONG, S., NÜSSLEIN, K., MARSH, T.L. AND FLYNN, S.J. (1999) Opening the black box of soil microbial diversity. *Applied Soil Ecology*, **13**, 109-122.

TILLMAN, R.W., SCOTTER, D.R., WALLIS, M.G. AND CLOTHIER, B.E. (1989) Water-repellency and its measurement by using intrinsic sorptivity. *Australian Journal of Soil Research*, **27**, 637-644.

TILMAN, D. (1999) The ecological consequences of changes in biodiversity: A search for general principles. *Ecology*, **80**, 1455-1474.

TIPPKÖTTER, R., EICKHORST, T., TAUBNER, H., GREDNER, B. AND RADEMAKER, G. (2009) Detection of soil water in macropores of undisturbed soil using microfocus X-ray tube computerized tomography (μCT). *Soil and Tillage Research*, **105**, 12-20.

TISDALL, J.M., COCKROFT, B. AND UREN, N.C. (1978) The stability of soil aggregates as affected by organic materials, microbial activity and physical disruption. *Australian Journal of Soil Research*, **16**, 9-17.

TISDALL, J.M. AND OADES, J.M. (1982) Organic matter and water-stable aggregates in soil. *Journal of Soil Science*, **33**, 141-163.

TORSVIK, V., GOKSØYR, J. AND DAAE, F.L. (1990) High diversity in DNA of soil bacteria. *Applied and Environmental Microbiology*, **56**, 782-787.

TORSVIK, V. AND ØVREÅS, L. (2002) Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology*, **5**, 240-245.

TRAORÉ, O., GROLEAU-RENAUD, V., PLANTUREUX, S., TUBEILEH, A. AND BŒUF-TREMBLAY, V. (2000) Effect of root mucilage and modelled root exudates on soil structure. *European Journal of Soil Science*, **51**, 575-581.

TRAPPE, J. (1987) Phylogenetic and ecologic aspects of myco-trophy in the angiosperms from an evolutionary standpoint. *In*: SAFIR, G.R., (ED.) Ecophysiology of VA mycorrhizal plants. Boca Raton, Florida, USA: CRC Press, p 5-25.

TRESEDER, K.K. AND ALLEN, M.F. (2000) Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. *New Phytologist*, **147**, 189-200.

TROUGHT, M.C.T. AND DREW, M.C. (1980) The development of waterlogging damage in young wheat plants in anaerobic solution cultures. *Journal of Experimental Botany*, **31**, 1573-1585.

TULLER, M., OR, D. AND DUDLEY, L.M. (1999). Adsorption and capillary condensation in porous media: Liquid retention and interfacial configurations in angular pores. *Water Resource Research,* **35**, 1949–1964.

TURNAU, K., RYSZKA, P., GIANINAZZI-PEARSON, V. AND VAN TUINEN, D. (2001) Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in southern Poland. *Mycorrhiza*, **10**, 169-174.

VAN BAVEL, C. H. M. (1949) Mean weight diameter of soil aggregates as a statistical index of aggregation. *Soil Science Society of America Proceedings*, **14**, 20-23.

VANCE, E.D., BROOKES, P.C. AND JENKINSON, D.S. (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **19 (6)**, 703-707.

VAN DER HEIJDEN, M.G.A. (2002) Arbuscular mycorrhizal fungi as a determinant of plant diversity: In search of underlying mechanisms and general principles. In: VAN DER HEIJDEN, M.G.A. AND SANDERS, I.R. (EDS.) Mycorrhizal ecology. Springer, Berlin, p 243–265.

VAN DER HEIJDEN, M.G.A., BOLLER, T., WIEMKEN, A. AND SANDERS, I.R. (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology*, **79**, 2082-2091.

VAN DER HEIJDEN, M.G.A., KLIRONOMOS, J.N., URSIC, M., MOUTOGLIS, P., STREITWOLF-ENGEL, R., BOLLER, T., WIEMKEN, A. AND SANDERS, I.R. (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, **396**, 69-72.

VAN DER HEIJDEN, M.G.A., RINAUDO, V., VERBRUGGEN, E., SCHERRE, C., BÀRBERI, P. AND GIOVANNETTI, M. (2008) The significance of mycorrhizal fungi for crop productivity and ecosystem sustainability in organic farming systems. 16th IFOAM Organic World Congress, Modena, Italy. 16-20th June 2008. Available at: http://orgprints.org/12004/01/12004.pdf

VAN DER HEIJDEN, M.G.A., STREITWOLF-ENGEL, R., RIEDL, R., SIEGRIST, S., NEUDECKER, A., INEICHEN, K., BOLLER, T., WIEMKEN, A. AND SANDERS, I.R. (2006) The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytologist*, **172**, 739-752.

VANDERKOORNHUYSE, P., HUSBAND, R., DANIELL, T.J., WATSON, I.J., DUCK, J.M., FITTER, A.H. AND YOUNG, J.P.W. (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Molecular Ecology*, **11**, 1555-1564.

VANDENKOORNHUYSE, P., RIDGWAY, K.P., WATSON, I.J., FITTER, A.H. AND YOUNG, J.P.W. (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. *Molecular Ecology*, **12**, 3085-3095.

VAN ELSAS, J.D., TAM, L., FINLAY, R.D., KILLHAM, K. AND TREVORS, J.T. (2007) Microbial interactions in soil. *In:* VAN ELSAS, J.D., JANSSON, J.K. AND TREVORS, J.T. (EDS.) Modern soil microbiology. Taylor and Francis Group (CRC Press), p 177-210.

VAN GEET, M., SWENNEN, R. AND WEVERS, M. (2000) Quantitative analysis of reservoir rocks by microfocus X-ray computerised tomography. *Sedimentary Geology*, **132**, 25-36.

VEEN, B.W., VAN NOORDWIJK, M., DE WILLIGEN, P., BOONE, F.R. AND KOOISTRA, M.J. (1992) Root-soil contact of maize, as measured by a thin-section technique. III. Effects on shoot growth, nitrate and water uptake efficiency. *Plant and Soil*, **139**, 131-138.

VIEIRA, F.C.S. AND NAHAS, E. (2005) Comparison of microbial numbers in soils by using various culture media and temperatures. *Microbiological Research*, **160**, 197-202.

VIERHEILIG, H., COUGHLAN, A.P., WYSS, U. AND PICHÉ, Y. (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology*, **64**, 5004-5007.

VIERHEILIG, H., LERAT, S. AND PICHÉ, Y. (2003) Systemic inhibition of arbuscular mycorrhiza development by root exudates of cucumber plants colonized by *Glomus mosseae*. *Mycorrhiza,* **13**, 167-170.

VISSER, E.J.W., NABBEN, R.H.M., BLOM, C.W.P.M. AND VOESENEK, A.C.J. (1997) Elongation by primary lateral roots and adventitious roots during conditions of hypoxia and high ethylene concentrations. *Plant, Cell and Environment*, **20**, 647-653.

VOGEL, H.J. (1997) Morphological determination of pore connectivity as a function of pore size using serial sections. *European Journal of Soil Science*, **48**, 365-377.

WALKER, T.S., BAIS, H.P., GROTEWOLD, E. AND VIVANCO, J.M. (2003) Root exudation and rhizosphere biology. *Plant Physiology*, **132**, 44-51.

WALLACE, H.R. (1958) The influence of pore size and moisture content of the soil on the migration or larvae of the beet eelworm, *Heterodera schachtii* Schmidt. *Annual Applied Biology*, **46**, 74-85.

WALLIS, M.G. AND HORNE, D.J. (1992) Soil water repellency. *Advancements in Soil Science*, **20**, 91-146.

WARDLE, D.A., ZACKRISSON, O., HÖRNBERG, G. AND GALLET, C. (1997) The influence of island area on ecosystem properties. *Science*, **277**, 1296-1299.

WARNER, G.S., NIEBER, J.L., MOORE, I.D. AND GEISE, R.A. (1989) Characterizing macropores in soil by computed tomography. *Soil Science of America Journal,* **53,** 653–660.

WATERS, I., KUIPER, P.J.C., WATKIN, E. AND GREENWAY, H. (1991) Effect of anoxia on wheat seedlings. *Journal of Experimental Botany*, **42**, 1427-1435.

WERTZ, S., DEGRANGE, V., PROSSER, J.I., POLY, F., COMMEAUX, C., FREITAG, T., GUILLAUMAUD, N. AND LE ROUX, X. (2006) Maintenance of soil functioning following erosion of microbial diversity. *Environmental Microbiology*, **8**, 2162-2169.

WEST, Y.O. AND POST, W.M. (2002) Soil organic carbon sequestration rates by tillage and crop rotation: A global data analysis. *Soil Science Society of America Journal*, **66**, 1930-1946.

WHEATON, A.D., MCKENZIE, B.M. AND TISDALL, J.M. (2008) Management to increase the depth of soft soil improves soil conditions and grapevine performance in an irrigated vineyard. *Soil and Tillage Research*, **98**, 68-80.

WHITE, R.E. (2006) Principles and practice of soil science: The soil as a natural resource. Fourth Edition. Blackwell Publishing, UK. Pp 363.

WIERSUM, L.K. (1957) The relationship of the size and structural rigidity of pores to their penetration by roots. *Plant and Soil*, **9**, 75-85.

WILLIAMS, B.S., GREENLAND, D.J., LINDSTROM, S.R. AND QUIRK, J.P. (1966) Techniques for the determination of soil aggregates. *Soil Science*, **101**, 157-163.

WOLFE, B.E., MUMMEY, D.L., RILLIG, M.C. AND KLIRONOMOS, J.N. (2007) Small-scale spatial heterogeneity of arbuscular mycorrhizal fungal abundance and community composition in a wetland plant community. *Mycorrhiza*, **17**, 175-183.

WRIGHT, D.A., KILLHAM, K., GLOVER, L.A. AND PROSSER, J.I. (1995) Role of pore size location in determining bacterial activity by protozoa in soil. *Applied and Environmental Microbiology*, **61**, 3537-3543.

WRIGHT, S.F. AND UPADHYAYA, A. (1996) Extraction of an abundant and unusual protein from soil and comparison with hyphal protein from arbuscular mycorrhizal fungi. *Soil Science*, **161**, 575-586.

WRIGHT, S.F. AND UPADHYAYA, A. (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, **198**, 97-107.

WU, J., JOERGENSEN, R.G., POMMERENING, B., CHAUSSOD, R. AND BROOKES, P.C. (1990) Measurement of soil microbial biomass C by fumigation-extraction – An automated procedure. *Soil Biology and Biochemistry*, **8**, 1167-1169.

WU, Q.-S., XIA, R.-X. AND ZOU, Y.N. (2008) Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *European Journal of Soil Biology*, **44**, 122-128.

YACHI, S. AND LOREAU, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 1463-1468.

YAO, H., HE, Z., WILSON, M.J. AND CAMPBELL, C.D. (2000) Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. *Microbial Ecology,* **40**, 223–237.

YAO, Q., WANG, L.R. AND CHEN, J.Z. (2009) Effect of arbuscular mycorrhizal fungal inoculation on root system architecture of trifoliate orange (*Ponciruc trifoliate L. Raf*.) seedlings. *Scientia Horticulturae*, **121**, 458-461.

YODER, R. (1936) A direct method of aggregate analysis of soils and a study of the physical nature of erosion losses. *Journal of the American Society of Agronomy*, **28**, 337-435.

YORK, C.A. AND CANAWAY, P.M. (2000) Water repellent soils as they occur on UK golf greens. *Journal of Hydrology*, **231-232**, 126-133.

YOUNG, I.M. (1998) Biophysical interactions at the root-soil interface: A review. *Journal of Agricultural Sciences*, **130**, 1-7.

YOUNG, I.M. AND CRAWFORD, J.W. (1998) Protozoan life in a fractal world. *Protist*, **152**, 123-126.

YOUNG, I.M. AND CRAWFORD, J.W. (2004) Interactions and self-organisation in the soil-microbe complex. *Science*, **304**, 1634-1637.

YOUNG, I.M. AND RITZ, K. (2000) Tillage, habitat space and function of soil microbes. *Soil and Tillage Research*, **53**, 201-213.

ZAK, J.C., WILLIG, M.R., MOORHEAD, D.L. AND WILDMAN, H.G. (1994) Functional diversity of microbial communities: a quantitative approach. *Soil Biology and Biochemistry*, **26**, 1101-1108.